Investigating the effects of nutrient concentration and light intensity on benthic biofilm development and phytoplankton growth in UK rivers.

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Declaration

While registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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Abstract

Phosphorus is often considered the limiting nutrient in rivers, and reducing concentrations has long been a key policy focus. However, experience has shown that many phosphorus mitigation schemes fail to achieve any improvements in ecological status. The primary aim of this thesis is to identify target phosphorus concentrations that need to be attained to improve river ecology.

In-stream flume mesocosms were deployed on three UK rivers of varying levels of nutrient enrichment. Phosphorus concentrations were simultaneously increased and decreased (by iron-dosing) to provide a concentration gradient over which periphyton accrual was examined. This identified whether rivers were phosphorus limited, and allowed a phosphorus-limiting threshold to be quantified. The effects of nitrogen, and combined phosphorus and nitrogen addition were also examined.

The River Lambourn study simultaneously manipulated light intensity and nutrient concentrations, demonstrating shading to have benefits in improving periphyton quality as a food resource and in reducing periphyton accrual. The near-pristine River Rede showed that increase in river phosphorus concentration had no effect on periphyton growth rate, but that there was a 3.5-fold increase in growth rate when phosphorus and nitrogen were added simultaneously, demonstrating the presence of sequential nutrient co-limitation. By repeating a 2005 study on the River Frome, the 2012 study proved that phosphorus-limiting thresholds change in response to changing river nutrient concentrations. Examination of changes in community structure by the trophic diatom index and flow cytometry provide evidence for a lower ecological threshold in rivers of *ca.* 30 µg l^{-1}.

A fast repetition rate fluorometer was used to assess phytoplankton stress across the Thames catchment throughout an algal bloom and data was examined alongside water quality data. Bloom development and collapse was primarily controlled by residence time and, secondarily, phosphorus concentration. This thesis has challenged traditional beliefs that phosphorus is the limiting nutrient in freshwater ecosystems and has provided insights on how to best meet the requirements of the Water Framework Directive and improve ecological status.

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Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of Tables

Chapter 5

Chapter 7

Appendicies

Abbreviations

AFDM – Ash free dry mass

AI – Autotrophic index

AM - Ashed mass

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

CEH – Centre for Ecology and Hydrology

DM – Dry mass

DOC – Dissolved organic carbon

EQR – Ecological quality ratio

eTDI – Expected trophic diatom index

F_m – Maximum fluorescence

F_0 – Minimum fluorescence

F_v – Variable fluorescence

NDS – Nutrient diffusing substrata

NSA's – Nitrate sensitive areas

NVZ's – Nitrate vulnerable zones

OD – Optical density

PP – Particulate phosphorus

PS I – Photosystem I

PS II – Photosystem II

SRP - Soluble reactive phosphorus

STW – Sewage treatment works

SUP – Soluble unreactive phosphorus

TC – Total carbon

TDI – Trophic diatom index

TDN – Total dissolved nitrogen

TDP – Total dissolved phosphorus

TN – Total nitrogen

TP – Total phosphorus

UKTAG – United Kingdom Technical Advisory Group for the Water Framework Directive

UWWTD – Urban Wastewater Treatment Directive

WFD – Water Framework Directive

WMS – Weighted mean sensitivity

Chapter 1: Introduction

The ecological status and water quality of rivers within the United Kingdom (UK) is of major environmental concern. Increasing industrialisation and agricultural intensification throughout the nineteenth and twentieth centuries have led to wide scale environmental degradation, as rivers were often used as 'dumping grounds' for effluent, industrial waste and other pollutants. Improving and maintaining ecological status and water quality of rivers is arguably one of the greatest environmental challenges to face as we proceed through the twenty first century.

Recent European-driven legislation in the form of the Nitrates Directive (Council of European Communities, 1991a), Urban Wastewater Treatment Directive (UWWTD) (Council of European Communities, 1991b) and Water Framework Directive (WFD) (Council of European Communities, 2000) is seeking to improve the water quality of Europe's rivers. The UK implementation of the WFD requires all inland water bodies in the UK to achieve 'good' ecological and chemical status by 2015. Specific indices have been developed to assess this for chemical water quality (UKTAG, 2008, UKTAG, 2013a) and ecological status of individual components of the river ecosystem, including the fish community (multi-metric fish index) (Coates *et al.*, 2007), macrophytes (mean trophic rank and LEAFPACS) (Holmes *et al.*, 1999), invertebrates (RIVPACS, ecological quality index and average score per taxa) (Clarke *et al.*, 2003) and diatoms (trophic diatom index (TDI) and diatom assessment of river ecological status) (Kelly *et al.*, 2001).

Current knowledge only goes as far as to define wide-ranging categories for trophic state (Dodds *et al.*, 1998). In their commentary, Neal and Jarvie (2005) raise a number of issues that the UK needs to address in order to successfully meet the requirements of the WFD; the first of these is "are there threshold concentrations for nutrient limitation in rivers?" (Neal and Jarvie, 2005). Without specific knowledge-based targets (Dodds *et al.*, 2010) it is difficult to know to what concentration nutrients need to be reduced to, to achieve 'good' status as specified by the WFD. The research carried out in this thesis examines factors affecting ecological status

and reviews current knowledge before undertaking a series of in-stream flume mesocosm experiments to answer Neal and Jarvie's question and quantify a phosphorus-limiting threshold for rivers in the UK. In applying this threshold, it will be possible to have the greatest ecological improvement for the least economic cost, by better targeting nutrient reduction measures.

1.1 UK nutrient targets

It is widely believed that improvements in ecological status of a river will follow from chemical improvements in its water quality. This belief is reflected in that the UK nutrient standards and classifications are based on sites that already have good or high ecological status for plant communities (as indicated by diatoms) (UKTAG, 2008). Nutrient concentrations in rivers have been seen as a key target for reduction, in order for the UK to meet the requirements of the WFD. The United Kingdom Technical Advisory Group for the WFD (UKTAG) is a multidisciplinary partnership of conservation and environmental agencies that are tasked with developing standards and targets for all aspects of the UK's water environment (coasts, estuaries, lakes and rivers), to underpin the implementation of the WFD. Specific water quality standards for good chemical status were first developed by UKTAG in 2008 and, for river environments, covered biological oxygen demand, dissolved oxygen concentration, pH and phosphorus concentration. The phosphorus standards for good chemical status (based on alkalinity and altitude) are given in Table 1.1. These were derived from 90 % of sites with good ecological quality (UKTAG, 2008).

Table 1.1: Phosphorus standards (annual mean soluble reactive phosphorus in µg l^{-1}) determined by the United Kingdom Technical Advisory Group for the Water Framework Directive to achieve 'good' status in rivers (UKTAG, 2008).

Alkalinity (mg l^{-1} of $CaCO_3$)	Altitude (m)	Annual mean SRP standard (µg l^{-1})
Low (< 50)	High (> 80)	40
Low (< 50)	Low (< 80)	50
High (> 50)	High (> 80)	120
High (> 50)	Low (< 80)	120

There has been controversy into the accuracy of these standards (Table 1.1) with subsequent investigations indicating that phosphorus concentrations are too high to achieve 'good' ecological status. For example, when examining a long-term data record for the River Kennet, Neal *et al.* (2010) found that soluble reactive phosphorus (SRP) concentrations had been reduced (due to improvements in sewage treatment) and were now below the good status standard, but that this was not coupled with an ecological improvement (in terms of a reduction in phytoplankton chlorophyll-*a* concentration).

The lack of ecological response in the River Kennet, despite meeting the 'good chemical' standard, clearly suggests that the standards are greater than the phosphorus-limiting threshold (see Section 1.2). This realisation is reflected in the recent proposed reductions in river phosphorus standards by the UKTAG to the annual average concentrations presented in Table 1.2 (UKTAG, 2013a). There was a need to revise standards based on recognition of the mismatch between phosphorus concentration and biology and also due to changes in biological standards (on which phosphorus standards were based) (UKTAG, 2013b). Although the new standards propose much lower phosphorus concentrations (Table 1.1 and Table 1.2) they must be used with caution: despite use of both the new phosphorus (UKTAG, 2013a) and biology (UKTAG, 2013b) standards, it is still believed that there is a mismatch between nutrient concentration (chemical status) and biology (ecological status) of up to 62 % across UK river sites (UKTAG, 2013a). There is great uncertainty about the direct relationship between river phosphorus concentration and biological response. For example, of the 804 UK river sites examined under the new

phosphorus and biology standards, ecological status (biology) was worse than expected based on phosphorus classification at 39 % of sites and better than expected at 23 % of sites (UKTAG, 2013a). Further experimental work is needed to reduce this mismatch and provide accurate quantitative chemical thresholds for ecological improvement in rivers across the UK.

Table 1.2: Proposed revised phosphorus standards (annual mean soluble reactive phosphorus in µg l^{-1}) to achieve 'good' status in UK rivers. Standards are based on regression analysis and numbers presented are the median of each class. Numbers in brackets are the upper and lower 5th and 95th percentile respectively (UKTAG, 2013a).

Alkalinity (mg l^{-1} of $CaCO_3$)	Altitude (m)	Annual mean SRP standard (µg l^{-1})
Low (< 50)	High (> 80)	28 (28 – 41)
Low (< 50)	Low (< 80)	40 (28 – 52)
High (> 50)	High (> 80)	48 (28- 70)
High (> 50)	Low (< 80)	69 (52 – 91)

1.2 Nutrient thresholds and research aims

The reasoning behind many of the nutrient limitation experiments conducted (see Section 1.10) is to determine the ecological (nutrient) threshold. Groffman *et al.* (2006) define an ecological threshold as "the point at which there is an abrupt change in an ecosystem quality, property or phenomenon, or where small changes in an environmental driver produce large responses in the ecosystem." Dodds *et al.* (2010) defines a driver as an "abiotic or biotic change over time or space that influences ecological state."

One of the main aims of this thesis is to define a phosphorus-limiting threshold (sometimes referred to as the phosphorus saturation point) for periphyton growth in UK rivers by experimental manipulation. In other words, to determine the SRP concentration above which phosphorus is in excess for growth (where adding phosphorus will have no periphyton growth response), and below which phosphorus is limiting to periphyton growth (Figure 1.1). Below this phosphorus-limiting

threshold, the river can be said to be in the "desired state" (Groffman *et al.*, 2006), as the reduced availability of phosphorus will reduce the risk of excessive algal growth. Experimentally determining the phosphorus-limiting threshold has not been achieved previously and at the commencement of this work, there was evidence to suggest nutrient standards were greater than the phosphorus-limiting threshold (e.g. Neal *et al.*, 2010). The actual value of the phosphorus-limiting threshold concentration could well vary between rivers, as has been shown in previous work globally (Chambers *et al.*, 2012). The threshold will be dependent on a number of environmental factors including geology, historical and current land-use, as well as the influence of other factors known to affect periphyton growth (Mainstone and Parr, 2002 and see Section 1.8).

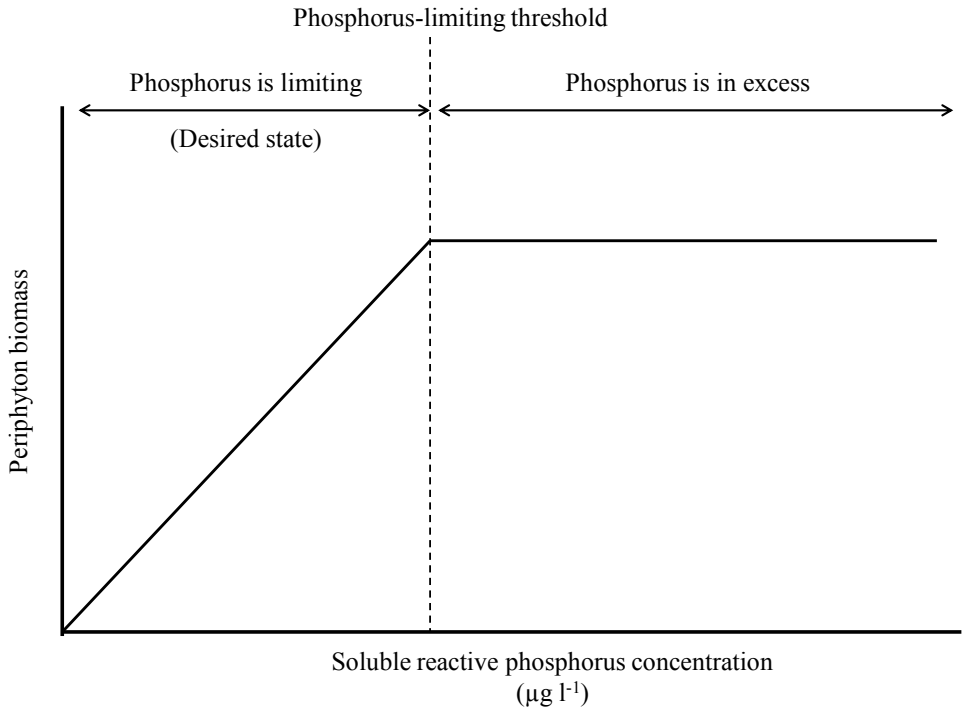


Figure 1.1: Schematic diagram of phosphorus uptake by periphyton showing the breakpoint where phosphorus becomes limiting to growth (phosphorus-limiting threshold). Drawn by the author.

Determining a knowledge-based threshold will complement existing broad ranging thresholds set as part of the requirements of the WFD (Mainstone and Parr, 2002, Neal and Jarvie, 2005, UKTAG, 2008). The work follows on from that of Bowes *et al.* (2007), Bowes *et al.* (2010) and Bowes *et al.* (2012a) in which experiments were run in three rivers with ambient SRP concentrations of 60, 120 and 225 µg l^{-1}. The results from these experiments found that, on each occasion, the ambient SRP concentration of the river was either at or above the phosphorus-limiting threshold. This was despite two of the rivers meeting the phosphorus standard at the time (UKTAG, 2008) and one of the rivers meeting the revised standard (UKTAG, 2013a).

By defining the phosphorus-limiting threshold, scientists and policy makers will be better informed when making decisions regarding catchment management and, therefore, be able to implement the most ecologically sound and cost effective solution to achieving improvements in ecological status and reduce the risk of algal blooms (Groffman *et al.*, 2006, Dodds *et al.*, 2010). If phosphorus concentration can be reduced and sustained below the phosphorus-limiting threshold, then an ecological response and improvement should be observed, which will help the UK comply with the ecological requirements of the WFD and other European legislation.

1.3 Macronutrients in the freshwater environment and their role

The two main nutrients needed for periphyton and macrophyte growth in the aquatic environment are phosphorus and nitrogen. Phosphorus and nitrogen are essential constituents of all cells, necessary for cell development and growth. Phosphorus is a component of adenosine triphosphate (ATP), a molecule which transports chemical energy around the organism for life processes including the synthesis of macromolecules and proteins. Phosphorus is also essential in the phospholipid bilayer of cell membranes and in maintaining the structure of genetic material including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nitrogen is needed for amino acids, which form proteins that are vital for growth and enzyme function in both flora and fauna. The process of photosynthesis, for example, is regulated by the D1 protein. Of phosphorus and nitrogen, nitrogen is usually the

most abundant. Phosphorus is present at much lower naturally-occurring concentrations, and is therefore often considered limiting to primary productivity in the freshwater environment. Consequently, it has the greatest potential to limit periphyton and macrophyte growth (Horne and Goldman, 1994, Mainstone and Parr, 2002).

Often cited as the main factor affecting periphyton accrual and biomass, nutrient concentrations have been credited with explaining between 23 and 40 % of variation in chlorophyll-*a* concentration / periphyton growth in streams (Biggs, 2000, Dodds *et al.*, 2002). Horner *et al.* (1983) also found that where periphyton biomass reached nuisance levels (150 mg m^{-2}), nutrient enrichment was present. The importance of nutrients in controlling aquatic ecosystems is evident in that water body classification (in terms of trophic status) is often based solely on nutrient concentrations (Dodds *et al.*, 1998, Gold and Sims, 2005).

1.4 Nutrient sources

1.4.1 Phosphorus

Naturally occurring processes that allow phosphorus to enter the freshwater environment include weathering of catchment soils and rocks, breakdown of biological material, input from direct precipitation, and desorption from minerals. Organic polyphosphate (chain structures) and metaphosphate (ring structures) are produced as a result of biological activity and in-stream processes. In the environment, phosphorus is most commonly present as phosphate ions (PO_3^-). High phosphorus concentrations in household detergents and sewage effluent make sewage treatment works (STW) major point sources of phosphorus, ultimately resulting in elevated phosphorus (especially SRP) concentrations in waters receiving effluent (Mainstone and Parr, 2002, Neal *et al.*, 2005, Jarvie *et al.*, 2006), a phenomenon that is not just restricted to rivers in urban areas (Jarvie *et al.*, 2006, Bowes *et al.*, 2009a).

The introduction of tertiary treatment at STW by so called ‘phosphorus-stripping’ has been shown to reduce within-river phosphorus concentration by up to 95 %

(Reynolds and Davies, 2001). This was illustrated in the Hogsmill River, a tributary of the River Thames (Millier *et al.*, 2010), where mean SRP concentration (adjusted for flow) upstream of the STW was 190 µg l^{-1}, while downstream SRP increased 10-fold to 1780 µg l^{-1}. After the introduction of phosphorus-stripping in April 2008, downstream mean SRP (adjusted for flow) was reduced by 68 % to 570 µg l^{-1}.

Figure 1.2 shows the proportion of total phosphorus (TP) and SRP that is estimated to enter rivers from common sources in Great Britain in 2004 (White and Hammond, 2009). Despite widespread tertiary treatment, household waste (including detergents and STW effluent) contributed the largest proportion to SRP concentrations (83, 55 and 78 % in England, Scotland and Wales respectively). Household waste was also the largest contributor to TP concentration in England and Scotland (78 and 54 % respectively). However, in Wales, household waste and agriculture made approximately equal contributions to total TP load (49 and 45 % respectively) (Figure 1.2).

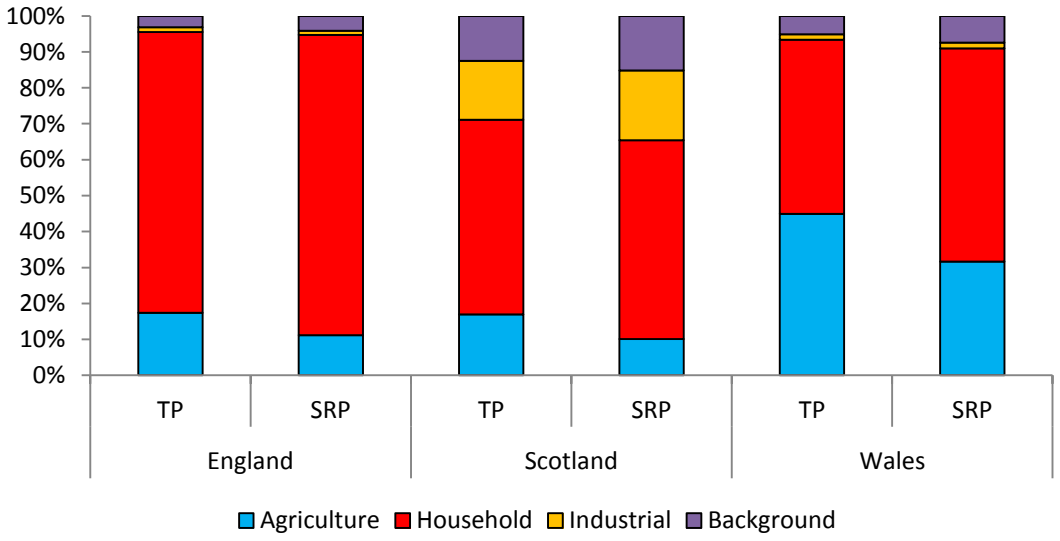


Figure 1.2: Proportion of total phosphorus (TP) and soluble reactive phosphorus (SRP) from common sources to total load in Great Britain. Data from White and Hammond (2009).

Phosphorus associated with agriculture is a common non-point (diffuse) source to rivers from overuse of fertilisers, increased livestock stocking rates and by direct excretion by animals into water courses (Carpenter *et al.*, 1998, Hooda *et al.*, 2000). Fertilisers and manure are rich in phosphorus and other nutrients, including nitrogen, needed for periphyton growth. Other non-point phosphorus sources include road run-off, septic tank input, seepage from agricultural slurry stores / animal housing and forestry plantations, as a result of fertiliser use and increased soil erosion / surface run off as a result of deforestation (Hooda *et al.*, 2000, Mainstone and Parr, 2002, Withers and Jarvie, 2008). Scientific evidence suggests the most effective way to reduce phosphorus concentrations in freshwater ecosystems is by simultaneously reducing fertiliser inputs and STW discharge consents (Whitehead *et al.*, 2013). In addition to the sources pictured in Figure 1.2, phosphorus accumulated in sediment can be a major source to river environments (due to sorption of phosphorus from the water column to the river bed sediments) (Owens and Walling, 2002, House, 2003), especially to rooted macrophytes. Thus, accumulated phosphorus in sediments may continue to be a problem for many years after the effect of external phosphorus inputs are reduced (Holtan *et al.*, 1988).

Due to the flowing nature of rivers and constant changes in sewage and agricultural inputs (which are often weather dependent), phosphorus concentrations in the water column are continually changing as a result of biological uptake and degradation (release) and longitudinal movement. The extent of biogeochemical cycling is largely dependent on catchment hydrology, reactivity of soils and sediments, and weather conditions (House, 2003). Biogeochemical cycling results in phosphorus constantly moving between the biotic and abiotic components of the aquatic ecosystem, a concept commonly referred to as 'nutrient spiralling' (Newbold *et al.*, 1981, Newbold *et al.*, 1983). Chemical and physical processes have been shown to have a key role in modifying and controlling phosphorus fluxes within the aquatic environment (House, 2003). The movement of phosphorus between different components in the aquatic ecosystem is visualised in Figure 1.3.

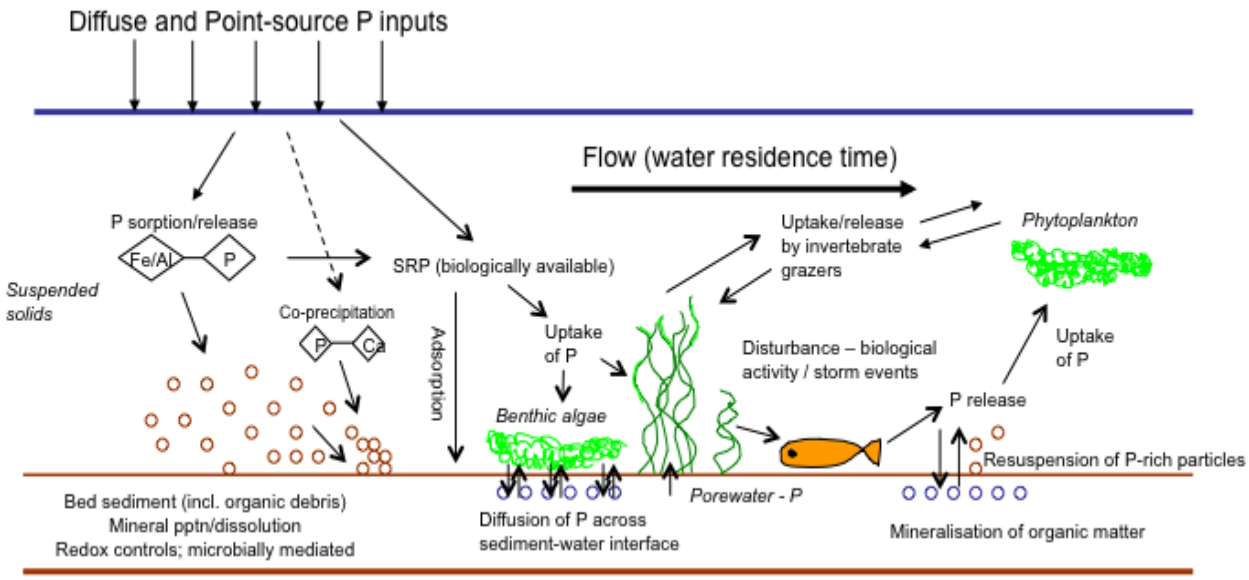


Figure 1.3: Schematic diagram illustrating how phosphorus inputs to rivers are continuously cycling between biotic and abiotic components of the ecosystem as water moves downstream. Diagram reproduced from Withers and Jarvie (2008).

1.4.2 Nitrogen

The most common forms of nitrogen in the aquatic environment are ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). All three forms are highly soluble, inorganic and bioavailable, meaning concentrations have the potential to affect periphyton biomass (Vitousek *et al.*, 1997). Like phosphorus, nitrogen can originate from point and non-point sources. The most dominant nitrogen source to rivers in England and Wales arises from agriculture (Figure 1.4) (Carpenter *et al.*, 1998, Hunt *et al.*, 2004), as a result of increased stocking rates of animals (leading to increased manure) and increased fertiliser use (six-fold increase since 1950) (Hooda *et al.*, 2000). The impact of these sources on river nitrogen concentrations is dependent on management practices such as timing and rate of manure application, as well as physical factors including slope gradient, soil type and climatic conditions (Hooda *et al.*, 2000). Through stable isotope analysis, Whitehead *et al.* (2002) found that 15 % of nitrogen applied to agricultural land as fertiliser was still available for biological uptake the following year. Other common sources are STW effluent, urban run-off and atmospheric deposition (as a result of industrialisation and fossil fuel

consumption) (Carpenter *et al.*, 1998). The proportion to the total nitrogen load from individual nitrogen sources in England and Wales is shown in Figure 1.4.

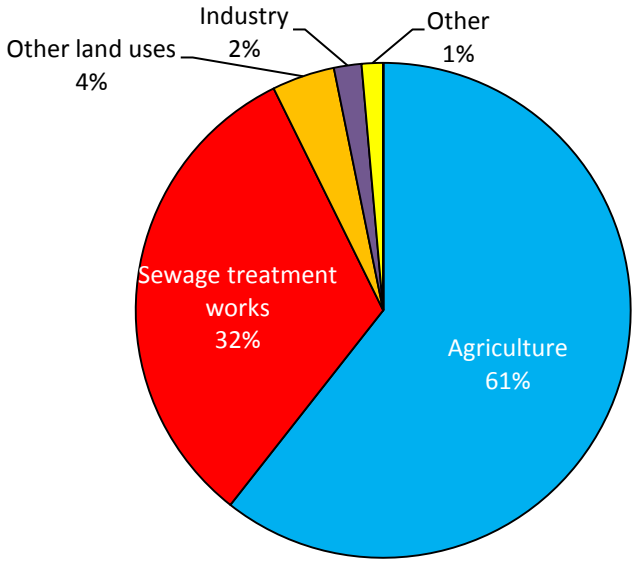


Figure 1.4: Pie chart showing proportion of different nitrogen sources to total nitrogen load in England and Wales in 2001/02. Other category includes septic tank discharges, combined storm overflow inputs and atmospheric deposition. Data from Hunt *et al.* (2004).

Nitrogen input to rivers has more than doubled since industrialisation (1800 AD), with global total mobilisable nitrogen (in the environment) increasing from 2 MT N km^{-2} yr^{-1} to 5 MT N km^{-2} yr^{-1} (Vitousek *et al.*, 1997, Green *et al.*, 2004). The largest increases in total nitrogen concentration have been observed in temperate climate zones including Europe and North America (Green *et al.*, 2004). The River Thames is one example of a rare long-term nitrogen dataset with continuous measurements at Hampton, near London for 140 years (from 1868 to 2008), allowing changes in concentration over time to be analysed (Howden *et al.*, 2010a). Prior to World War Two, nitrate concentrations remained at approximately 2 mg l^{-1} - N. Post-war agricultural intensification had a direct effect on nitrogen concentration, causing it to double to 4 mg l^{-1} between 1945 and 1970. A second doubling was observed (concentrations of 8 mg l^{-1} - N) in the early 1970s and was attributed to an increase in

inorganic fertiliser use. Due to changes in land management in the late1980s / early 1990s (induced by the introduction of the nitrates directive (Council of European Communities, 1991a)), the rising trend in increased nitrogen concentrations has been halted in recent years and concentrations have levelled off. However, these remain high with average concentrations of 8 mg l^{-1} - N (Howden *et al.*, 2010a).

The introduction of the Nitrates Directive within the UK (91/676/EC) (Council of European Communities, 1991a), led to the early establishment of Nitrate Sensitive Areas (NSA's) which then developed into Nitrate Vulnerable Zones (NVZ's), with the aim of protecting both surface and ground waters and drinking water supplies. NVZ's are designated where run-off from agricultural land contributes to nitrate concentrations measured in polluted waters, and agriculture contributes more than 20 % to the total nitrogen load. Streams and rivers are typically considered to be polluted if the nitrate concentration measured is greater than the drinking water standard of 50 mg l^{-1} NO_3 (Drinking Water Inspectorate, 2000). To be compliant in areas classified as NVZ's, farmers must meet a number of statutory requirements including calculating nitrogen mass balances for crops (to prevent overuse of fertiliser), producing risk maps of where manure is spread, keeping accurate records of the limited quantities of nitrogen allowed to be applied to the land and the restricted timing of applications, and provide adequate and safe storage for livestock manure.

The establishment and maintenance of NVZ's has come at a significant cost, yet, in their analysis of the effectiveness of NVZ's some 15 years after creation, Worrall *et al.* (2009) found there to be no overall statistically significant reduction in nitrogen concentration. Although 29 % of NVZ's, analysed showed an improvement in water quality over a minimum 12 year period in comparison to control catchments, 31 % showed a significant decrease in water quality (in comparison to control catchments). Furthermore, 69 % of NVZ's analysed showed no significant improvement in water nitrogen concentration. Despite this, a process-based model derived by the British Geological Survey has suggested that peak nitrogen concentrations may have already been achieved in many (non-chalk) aquifers across the UK (Wang *et al.*, 2012). As such, nitrogen concentrations in coming decades may well decrease, which would

ultimately result in a decrease in periphyton biomass in streams and rivers and an overall improvement in ecological status

Due to its high solubility, nitrate often leaches into groundwater supplies which can greatly increase river nitrate concentrations (Smith *et al.*, 2010). Nitrogen leaching is a particular concern in the rare chalk stream habitat found in southern England. In chalk environments, nitrate is stored in the unsaturated zone within the chalk aquifer and contributes a significant concentration of nitrogen to the groundwater baseflow component of the stream, even when other nitrogen sources are reduced (Howden and Burt, 2008). For 60 % of the chalk aquifer across the UK, peak nitrate input to streams is predicted not to be reached for many decades (Wang *et al.*, 2012). In a similar way to phosphorus, stored nitrate can continue to contribute to stream nitrogen concentrations for several decades after reductions in sources (from changes in land management). In the aquifers of the Thames basin, for example, the lag time has been calculated to be *ca.* 30 years (Howden *et al.*, 2011).

1.4.3 Seasonality of nutrient concentrations

Rivers dominated by constant point sources of nutrients (e.g. STW effluent) tend to have higher nutrient concentrations in the summer, as reduced water flow leads to lower dilution. This period coincides with the main macrophyte and periphyton growing season, often leading to periphyton blooms and an increased eutrophication risk (see Section 1.6) (Hilton *et al.*, 2006, Jarvie *et al.*, 2006). Conversely, non-point source dominated rivers tend to have higher nutrient concentrations in autumn / winter as a result of higher rainfall leading to increased surface run-off. Load-apportionment models have been developed to quantify the proportion of point and non-point sources to total nutrient load in rivers using these different flow response characteristics (Bowes *et al.*, 2008, Bowes *et al.*, 2010b, Greene *et al.*, 2011).

1.5 Phosphorus speciation and bioavailability

Phosphorus in the freshwater environment may be present in the colloidal, particulate or dissolved form (Reynolds and Davies, 2001). The amount present is

complex due to there being a number of different chemical forms with different levels of bioavailability. Biologically available (bioavailable) phosphorus is defined as the fraction that is readily assimilated by organisms or is made more available by actions of the organisms themselves (e.g. by production of phosphatase enzymes) and includes intracellular phosphorus, that has already been assimilated (Reynolds and Davies, 2001). The situation regarding phosphorus speciation is further complicated by different authors using different terminology to describe similar chemical fractions (Haygarth and Sharpley, 2000). To avoid confusion, the definitions of the five fractions used in this thesis are presented below.

Soluble reactive phosphorus (SRP) – the filtered phosphorus fraction comprised of truly dissolved and colloidal material less than 0.45 µm, and includes fully dissociated inorganic orthophosphate ions including di-hydrogen phosphate ($H_2PO_4^-$), mono-hydrogen phosphate (HPO_4^{2-}) and phosphate (PO_4^{3-}). SRP is measured using the molybdate blue reaction (see Section 2.4.2) (Murphy and Riley, 1962) and because of this is sometimes referred to as molybdate-reactive phosphorus. Other common terms for SRP include dissolved reactive phosphorus, filterable reactive phosphorus and dissolved inorganic phosphorus (Haygarth and Sharpley, 2000). SRP is the most readily bioavailable form of phosphorus in the freshwater environment (Reynolds and Davies, 2001). Consequently, concentrations of SRP are of greatest concern in terms of water quality and eutrophication risk, especially during the summer growing season.

Total dissolved phosphorus (TDP) – this fraction is also filtered (0.45 µm) and consists of inorganic species plus soluble organic phosphorus compounds. During analysis, persulphate acid digestion of the sample (Mackereth *et al.*, 1989) converts the organic phosphorus compounds to soluble inorganic phosphates which can then be quantified spectrophotometrically by reaction with molybdate (Murphy and Riley, 1962).

Soluble unreactive phosphorus (SUP) – the fraction calculated by subtracting the SRP concentration from the TDP concentration. SUP is also known as dissolved hydrolysable phosphorus and dissolved organic phosphorus. SUP can become bioavailable as a result of enzymatic hydrolysis (Holtan *et al.*, 1988).

Total phosphorus (TP) – the fraction quantified by persulphate acid digestion of an unfiltered water sample, and consists of inorganic, organic and particulate-bound phosphorus (Mackereth *et al.*, 1989).

Particulate phosphorus (PP) – the fraction calculated from TP minus TDP. PP is equivalent to the fraction of phosphorus sorbed to particulates.

1.6 Eutrophication

Eutrophication is a term used to describe nutrient enrichment and the processes that occur in aquatic and terrestrial ecosystems as a result. Nitrogen and phosphorus are the key drivers to the eutrophication process (Gold and Sims, 2005). The extent of the eutrophication problem has accelerated in recent decades due to rapid increases in anthropogenic nutrient inputs described in Section 1.4 and in Mainstone and Parr (2002). Eutrophication is a particular concern for ecological quality and status when high nutrient concentrations coincide with the spring / summer plant (including periphyton) growing season (Jarvie *et al.*, 2006).

Excess nutrients can result in increased macrophyte and periphyton growth as well as an increase in planktonic algae suspended in the water column (Smith, 2003), ultimately leading to an imbalance between the macrophyte and periphyton community (Mainstone and Parr, 2002). An increase in periphyton and planktonic algae can ultimately reduce or even eradicate macrophyte standing crops as they colonise and cover macrophyte leaves, thereby reducing light availability to the plant (Hilton *et al.*, 2006). There is often a shift in species composition from diatoms and nanoeukaryotes to an increase in bloom-forming species and cyanobacteria, which may be toxic to animals and humans and inedible to grazing invertebrates (Smith, 2003). A loss of macrophytes has knock-on effects for other components of the aquatic ecosystem, as it affects feeding of fish and invertebrates and leads to a loss of habitat and shelter. Other ecosystem effects include increased turbidity / decreased water clarity, loss of species diversity, shift in species composition, and reduced night-time dissolved oxygen concentrations that can ultimately lead to fish kills (Smith, 2003, Gold and Sims, 2005).

As well as the ecosystem effects described above, eutrophication can also result in social and economic problems. These include decreased aesthetic value of a water body (which can have knock-on economic effects including loss of recreational value) and taste, odour and filtration problems with drinking water supplies (Pretty *et al.*, 2002, Smith, 2003). In 2002, the estimated (total damage) cost of freshwater eutrophication in the UK was between £75 million and £114.3 million per year, with approximately £54.8 million being spent each year to address the effects of eutrophication (Pretty *et al.*, 2002). More recently, within the River Thames catchment alone, the cost of meeting the phosphorus standard required by the WFD has been estimated to be £40.6 million each year (Whitehead *et al.*, 2013).

1.7 Periphyton

The term 'periphyton' describes submerged aquatic communities that are attached to substrates. Periphyton can proliferate on a number of substrates in the freshwater environment including rocks, sand (and fine sediments), macrophytes and animals (Horne and Goldman, 1994). The term originates from the Greek 'peri' meaning around and 'phuton' meaning plant (Oxford English Dictionary, 2013). Periphyton communities are a complex mixture of algae (including diatoms), cyanobacteria, fungi, detritus and heterotrophic organisms (Horne and Goldman, 1994). Due to their rapid response and high sensitivity to environmental change, periphyton biofilms are often used as indicators of water quality worldwide (McCormick *et al.*, 1996, Vis *et al.*, 1998, Burns and Ryder, 2001). Within the UK, diatoms are used as one of the main ecological indicators of water quality in relation to nutrient concentrations and to assess whether requirements of the WFD have been met (through the TDI) (Kelly, 1998, Kelly *et al.*, 2001).

Periphyton communities can quickly develop to nuisance levels, leading to impairment of aquatic ecosystems. They have been identified as the key ecological component that drives the ecosystem degradation that can result from eutrophication (Hilton *et al.*, 2006). Much research has been conducted to determine at what point levels of periphyton becomes a 'nuisance' and upset the ecological balance of a system. Published thresholds range between 100 to 200 mg m^{-2} and can be based on

mean or maximum values (Welch *et al.*, 1988, Dodds *et al.*, 1997, Biggs, 2000) with values over 150 mg m^{-2} cited as causing a degradation in aesthetic quality (Welch *et al.*, 1988). Nutrients (especially phosphorus and nitrogen concentrations) are often implicated as the main factor affecting periphyton growth, as illustrated by the fact that Dodds *et al.* (1998) suggested a classification system for stream trophic state based upon phosphorus and nitrogen concentrations.

1.8 Other factors affecting periphyton growth

Although nutrients are often cited as the main factor controlling periphyton growth, a number of other environmental factors also affect the kinetics of periphyton growth and community structure. A study by Welch *et al.* (1992) found that actual periphyton biomass was only 35 % of that predicted by an ecological model, based on phosphorus concentration, velocity and temperature. In the 'real world,' it is often difficult to separate the effects of nutrients from other environmental variables (Mainstone and Parr, 2002) and periphyton communities are often limited by more than one environmental factor.

1.8.1 Light

Light is essential in regulating periphyton biomass in streams as it is essential to the process of photosynthesis. Modelling studies have shown that at constant nutrient concentrations there can be significantly less algae in shaded versus unshaded streams (Munn *et al.*, 2010). Predictions made in modelled data have been observed in the field in other experiments. Shading has been cited as causing a four to five-fold decrease in chlorophyll-*a* concentrations and ash free dry mass (AFDM) of river biofilms (Lowe *et al.*, 1986, Hill and Knight, 1988). More recently, Sturt *et al.* (2011) examined the effects of shading and invertebrate grazing in determining controllers of nuisance periphyton and found that both factors were capable of significantly reducing periphyton growth, leading to the conclusion that in shaded streams, periphyton productivity was reduced. Shading of a stream can have a strong bottom-up control on periphyton growth.

1.8.2 Flow velocity

Periphyton communities can exhibit a mixed response to increased flow velocity. Up to a certain point, increasing flow will lead to an increase in periphyton biomass as a nutrient gradient is maintained between cells and the water column. However, above this threshold, increased flow will result in hydrodynamic drag, leading to decreased immigration, sloughing and loss of periphyton biomass (Hilton *et al.*, 2006). Highest periphyton biomass has been shown to occur at intermediate flow velocities (0.10 to 0.20 m s^{-1}) (Stevenson, 1996). In addition to affecting overall biomass, flow velocity can have an indirect effect on periphyton communities by altering morphology (Stevenson, 1996) and nutrient dynamics (Bowes and House, 2001).

In their re-circulating flume experiment, Horner *et al.* (1983) suggest maintaining velocity above 0.15 m s^{-1} to avoid nuisance levels of periphyton accumulation. This agrees with work by Biggs and Gerbeaux (1993) who found algal biomass on natural substrates was highest at velocities between 0.10 and 0.20 m s^{-1}. A literature review by Stevenson (1996) showed strong linkages between flow velocity and nutrient concentration, suggesting that optimum flow velocity (for maintaining periphyton biomass) decreases as nutrient concentration increases.

1.8.3 Grazing

Invertebrate grazing has been shown to control periphyton biomass and community structure in a number of studies (Welch *et al.*, 1992, Bergey and Resh, 1994, Anderson *et al.*, 1999, Sturt *et al.*, 2011) and can be said to have a strong top-down control on total periphyton biomass. Whether or not grazing invertebrates have an effect on periphyton density is dependent on grazer density / consumption rate and grazer feeding morphology compared to that of the periphyton growth form (Steinman, 1996). Welch *et al.* (1992) have suggested a grazing density of over 3000 invertebrates m^{-2} must be maintained in order to keep summer periphyton biomass low. Approximately 70 % of studies examined by Feminella and Hawkins (1995) and 59 % of those examined by Hillebrand (2009) reported that grazing significantly reduced chlorophyll-*a* concentrations and AFDM of biofilms. The absence of grazers

has also been shown to have a stronger effect on periphyton biomass than the influence of nutrient concentration (Welch *et al.*, 1992, Hillebrand, 2002).

In addition to affecting overall abundance of periphyton, 81 % of the studies examined by Feminella and Hawkins (1995) found grazers to have an effect in structuring periphyton communities. The most common effect was a decrease in dominant diatom species, such as *Gomphonema*, *Melosira*, and *Nitzschia* sp. This coincided with an increase in grazing resistant periphyton species, including the chlorophyte *Stigeoclonium*, the diatoms *Achnanthes lanceolata* and *Cocconeis placentula*, and the cyanobacterium *Calothrix* (Feminella and Hawkins, 1995). Furthermore, grazing has been shown to alter nutrient cycling in periphyton biofilms by altering hydraulic characteristics within the biofilm (Steinman, 1996).

1.8.4 Temperature

Due to its importance in regulating biochemical reactions (including nitrogen fixation and other enzymatic reactions), the thermal regime of rivers has been cited as being critical in maintaining overall stream health (DeNicola, 1996, Caissie, 2006). Temperature is known to affect the kinetics of periphyton communities due to its interactions with photosynthesis and respiration. Growth rate of communities is known to increase as temperature increases. However, at temperature extremes (low and high), temperature can cause sudden decreases in growth thereby having a negative effect on kinetics (Talling, 2012). Furthermore, at higher temperatures, proteins and nucleic acids denature and photosystems degrade (Davison, 1991).

Generally, river temperature is controlled by the amount of solar radiation. However, at smaller scales, local contributions such as groundwater input can change the thermal regime of rivers (DeNicola, 1996). Interactions between temperature and nutrients have been previously cited (Lamberti and Resh, 1983, DeNicola, 1996, Marcarelli and Wurtsbaugh, 2006). When looking at nitrogen fixation by periphyton, Marcarelli and Wurtsbaugh (2007) concluded that periphyton metabolism was controlled by nutrients, but the magnitude of the response and growth kinetics were regulated by temperature.

Like flow velocity and grazing, temperature has been shown to affect the composition of periphyton communities. In a temperature manipulation experiment conducted by Patrick *et al.* (1969), communities were dominated by diatoms at lower temperatures (20 to 28 °C), green algae at intermediate temperatures (30 to 35 °C) and cyanobacteria at higher temperatures (35 to 40 °C). Temperature has also been shown to affect trophic interactions and the strength of top-down control of aquatic ecosystems (Kishi *et al.*, 2005). Periphyton communities have been shown to be resilient to high temperatures, with short recovery times (less than one year) once temperature stress was relieved (DeNicola, 1996).

1.9 Nutrient limitation

A strategy commonly employed to reduce the risk of excessive algal growth is nutrient limitation. The concept of limitation is not new and was first identified by Justus von Liebig (1840) in his Law of the Minimum which states that growth is controlled by the resource that is in least supply (not the total resources available) (Figure 1.5A - C).

In order to grow and reach their maximum potential, plants and algae need both macronutrients (e.g. phosphorus, nitrogen, carbon and oxygen) and micronutrients (e.g. copper, iron, manganese and zinc) as well as optimal conditions of light, flow and temperature (Mainstone and Parr, 2002). The Redfield ratio (based on the elemental composition of marine phytoplankton) states that the molar ratio of carbon: nitrogen: phosphorus necessary for biotic growth should be 106: 16: 1 (Redfield, 1958). Based on this, if the bioavailable nitrogen: phosphorus ratio is less than 16: 1, the limiting nutrient has traditionally been assumed to be nitrogen. If the bioavailable ratio is greater than 16: 1, phosphorus is considered limiting. Due to this belief, phosphorus has been considered to be the limiting nutrient in most freshwater ecosystems (Horne and Goldman, 1994), because nitrogen: phosphorus ratio for most anthropogenically impacted rivers are usually greater than 16: 1.

A recent review, however, has discredited the Redfield ratio, concluding that predictions of which nutrient is limiting cannot be based on relative or absolute

values of nitrogen and phosphorus in the water column (Keck and Lepori, 2012). A commentary by Jarvie *et al.* (2013) supports the idea that concentration of phosphorus in the water column is often not the cause of algal blooms and subsequent degradation in water quality. They explain that phosphorus mitigation measures do not always improve ecological status due to the complex, interrelated nature of freshwater ecosystems and suggest that future mitigation measures must take a more holistic approach. This supports the ideas of a review conducted by Elser *et al.* (2007) who examined 653 freshwater studies of nutrient limitation (alongside 243 marine and 173 terrestrial studies) and found that, in freshwater ecosystems, instances of nutrient co-limitation were significantly higher than limitation by phosphorus or nitrogen alone. A meta-analysis of nutrient diffusing substrata (NDS) experiments also showed that co-limitation by nitrogen and phosphorus in combination to be more common (23% of studies) than limitation by nitrogen (17 %) or phosphorus (18 %) alone (Francoeur, 2001).

1.9.1 Nutrient co-limitation

Nutrient co-limitation of a river system occurs when there is no biomass response to the addition of nutrients individually (A or B), but there is a biomass response when the same two nutrients are added simultaneously (AB - Figure 1.5D, strict co-limitation) (Elser *et al.*, 2009). However, the nature of co-limitation is complex, being complicated by multiple definitions, many of which have the same meaning (Figure 1.5). For example, strict co-limitation has also been referred to as simultaneous co-limitation (Harpole *et al.*, 2011).

In contrast to strict co-limitation, sequential co-limitation (Figure 1.5E and F) occurs when a system exhibits a biomass response to a single nutrient addition (A or B), but the response is greater when two nutrients are added simultaneously (AB) (Elser *et al.*, 2009). Sequential co-limitation has also been described as serial limitation (Harpole *et al.*, 2011) and synergistic limitation (Davidson and Howarth, 2007, Allgeier *et al.*, 2011) (Figure 1.5E and F). A third form of nutrient co-limitation, defined by Allgeier *et al.* (2011) as additive co-limitation is represented in Figure 1.5G. In this case the biomass response to the simultaneous addition of AB is equal

to the individual biomass response to A plus the individual biomass response to B. This type of co-limitation has also been defined as independent co-limitation (Allgeier *et al.*, 2011). Finally, antagonistic co-limitation (Allgeier *et al.*, 2011) occurs when the additive response from single addition of A and single addition of B is greater than the response of AB being added simultaneously (Figure 1.5H).

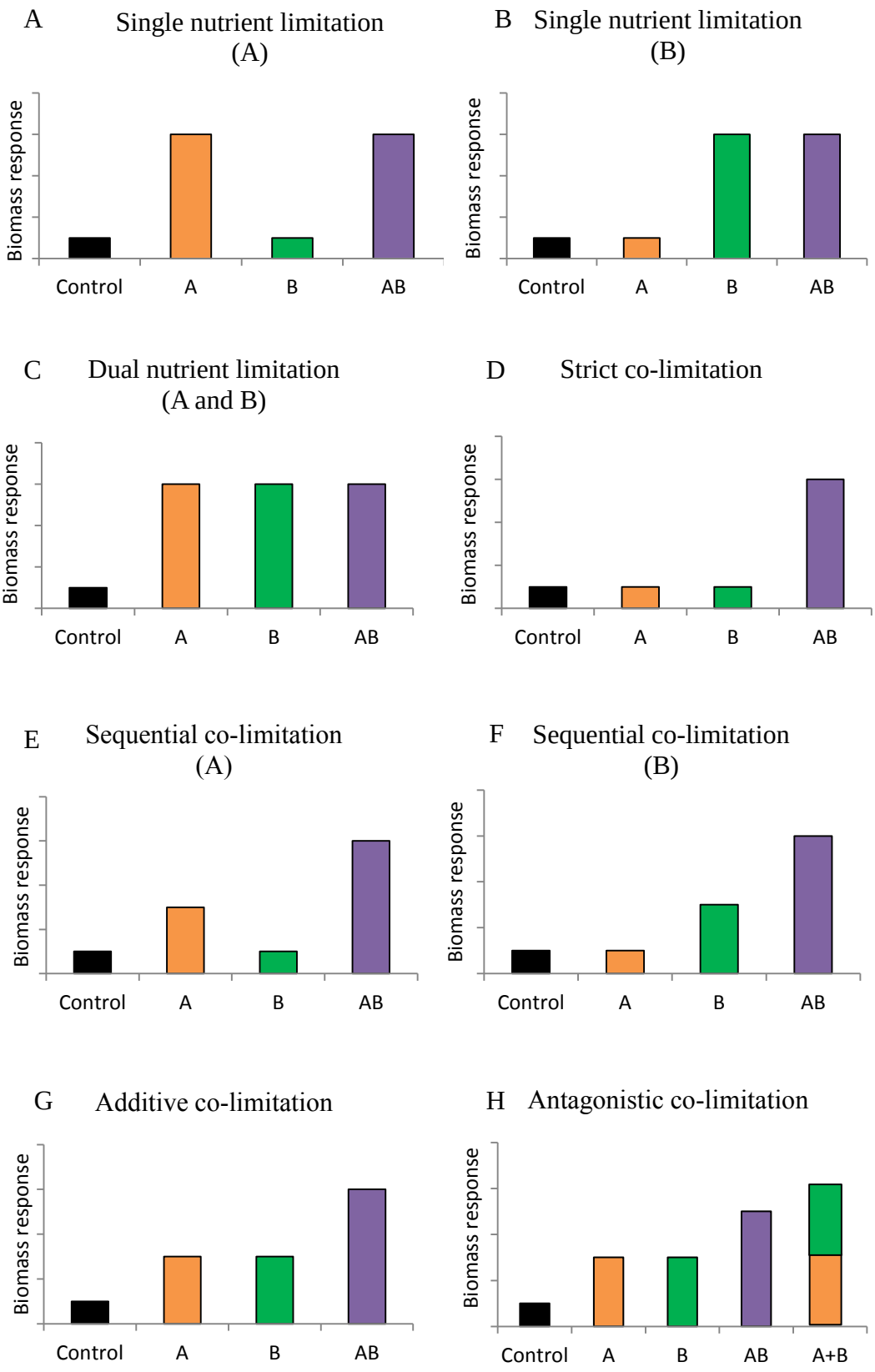


Figure 1.5: Conceptual diagram illustrating different forms of nutrient limitation. Drawn by the author.

1.10 Nutrient limitation experiments

Numerous studies have been undertaken to examine the effects of eutrophication and nutrient limitation on stream ecology in an attempt to define 'acceptable' nutrient concentrations. Such experiments increase phosphorus and / or nitrogen concentrations in order to examine whether or not there is an effect on periphyton biofilm growth. There are a number of ways of doing this, as described in detail below.

1.10.1 Direct stream enrichment

Direct stream enrichment involves artificially increasing the nutrient concentration in a stream reach and maintaining concentration at the increased level for a set time period. Artificial substrates are placed in the stream at the beginning of the experiment and the periphyton biofilm that grow on them are quantified and analysed at the end of the experiment. Substrates are often placed in an upstream control (non- modified reach) at the same time to allow comparisons to be made.

One of the first nutrient limitation experiments undertaken involved direct stream enrichment (fertilisation) (Huntsman, 1948) and a number of further studies have applied this approach (Elwood *et al.*, 1981, Perrin *et al.*, 1987, Peterson *et al.*, 1993). Direct stream enrichment is fairly easy to achieve. However, it can lead to wider scale enrichment, with elevated nutrient concentrations being recorded up to 2 km downstream of the study site (Perrin *et al.*, 1987). Thus, due to ethical concerns about the widespread ecological effects of direct stream enrichment and the potential to adversely affect other parts of the stream ecosystem, this method is less common than other nutrient limitation experiments. One way of assessing nutrient effects using this approach to study nutrient limitation, while causing minimal disturbance, is to investigate the response of periphyton communities upstream and downstream of sewage / industrial discharge inputs (Welch *et al.*, 1992, Millier *et al.*, 2010).

Studies of direct stream enrichment have indicated nutrient limitation of oligotrophic streams. Peterson *et al.* (1993) increased SRP concentration from a background level that was below detection limits (< 0.05 µmol l^{-1}) to a concentration of 0.32 µmol l^{-1}

(10 µg l^{-1}) and initially found a positive significant response in chlorophyll-*a* concentrations. Similarly, Perrin *et al.* (1987) increased SRP concentration of a coastal stream in British Columbia from a background concentration of 1 µg l^{-1} to a mean of 15 µg l^{-1} in one reach and a mean of 25 µg l^{-1} in a second reach, resulting in a 9 and 15-fold increase in chlorophyll-*a* concentration. After four weeks of enrichment from an ambient SRP concentration of 4 µg l^{-1} to treatment concentrations of 60 and 450 µg l^{-1}, Elwood *et al.* (1981) found significant increases in chlorophyll-*a* concentration compared to the control reach. There was, however, no significant difference between the two phosphorus enrichment treatments, suggesting that at a concentration of 60 µg l^{-1}, SRP concentration was above the phosphorus-limiting threshold.

1.10.2 Nutrient diffusing substrata

A number of studies have used nutrient diffusing substrata (NDS) and nutrient periphytometers to determine which nutrient is limiting (Chessman *et al.*, 1992, Matlock *et al.*, 1998, Francoeur *et al.*, 1999, Tank and Dodds, 2003, Godwin *et al.*, 2009, Johnson *et al.*, 2009, Lang *et al.*, 2012). NDS can be constructed out of plastic cups (Johnson *et al.*, 2009), clay (plant) pots (Chessman *et al.*, 1992, Godwin *et al.*, 2009) and other materials. Most contain agar solution that is enriched with either nitrogen, phosphorus or a combination of nitrogen and phosphorus.

NDS are deployed in different streams, generally alongside an un-enriched control and left for a specified amount of time so that nutrients can diffuse into the water and a periphyton biofilm can develop on the NDS surface (Capps *et al.*, 2011). NDS, therefore, provide a cost-effective and easily replicable method to determine nutrient limitation without the artificial nature of an enclosure. However, nutrient diffusion rates have been shown to vary widely between replicate NDS, partly due to differences in construction material and design (Capps *et al.*, 2011) and because pores can become blocked, preventing nutrient diffusion (Brown *et al.*, 2001). Furthermore, in flowing waters (i.e. streams and rivers) there is an exponential decrease in nutrient concentration with time (nutrients can be depleted within six days) and diffusion is affected by flow (Corkum, 1996a). There is also potential bias

in NDS experiments as Corkum (1996a) found that nitrogen was retained within the agar for longer than phosphorus. In their study analysing different types of terracotta, Brown *et al.* (2001) simply concluded that NDS do not work.

Despite their limitations, NDS remain a popular method of examining nutrient limitation. A meta-analysis conducted by Francoeur (2001) analysed 237 studies that used NDS as a means of assessing nitrogen and phosphorus limitation. The results showed that of the studies examined, 17 % showed nitrogen limitation, 18 % showed phosphorus limitation and nearly half (43 %) indicated no nutrient limitation. Godwin *et al.* (2009) deployed NDS at five sites with different land-uses along Spring Creek, Pennsylvania in different seasons to assess nitrogen limitation of periphyton. Statistical analysis showed no significant nutrient limitation in the river in terms of chlorophyll-*a* concentration or nutrient accumulation rates in any season. A study by Johnson *et al.* (2009) attempted to assess nutrient limitation on a large-scale deploying NDS across nine streams in eight different eco-regions. The results showed that for 75 % of sites on inorganic substrata and 65 % of sites on organic substrata, nutrients did not limit periphyton growth. The sites where nutrients were not limiting generally comprised of agricultural and urban land-uses whereas nearly all reference-condition streams (23 out of 34) displayed nutrient limitation.

1.10.3 Flume mesocosms

Flume mesocosms have been defined as constructed channels with controlled flow of water, which are used to study some physical, chemical or biological property of natural streams (McIntire, 1993). Mesocosms allow individual populations to be investigated at the same time as whole ecosystems (Odum, 1984), while providing better treatment control than in the natural environment. Flumes tend to fall into one of three categories, being either closed systems (with no water exchange), re-circulating systems (with partial water exchange) or open systems (with complete water exchange) (McIntire, 1993). The employment of mesocosms within streams is based upon the assumption that data obtained are realistic, so results are applicable to the natural environment (Petersen and Hastings, 2001). Mesocosms have been shown to provide a high degree of realism and physiochemical measurements made

within mesocosms have been shown to be similar to simultaneous measurements taken in the river channel (Harris, 2006).

The use of mesocosm experiments has become increasingly common in ecological studies in recent years and has been used as the primary means of investigation in a number of studies. These studies can be divided into two groups: those where flumes are located within the stream channel (Hart and Robinson, 1990); and (more commonly) those were streamside flumes are supplied with water from an adjacent stream (Stockner and Shortreed, 1978, Bothwell, 1985, Horner *et al.*, 1990, Mundie *et al.*, 1991, Stelzer and Lamberti, 2001, Rier and Stevenson, 2006, Davies and Bothwell, 2012, Wagenhoff *et al.*, 2013).

1.10.3.1 Portable in-stream flume mesocosms

Stream enrichment, NDS and flume studies are all based on nutrient additions to the river, and so can only investigate the effect of increasing nutrient concentrations on periphyton accrual rate. In this present period of WFD implementation, this is an unrealistic scenario, as most rivers across the UK and Europe will experience major reductions in nutrient concentrations, rather than increases. Studies that can only increase nutrient concentration can identify whether nutrients are currently limiting or in excess, but are unable to provide information on the concentration at which nutrients become limiting for rivers that are currently in excess.

The phosphorus-limiting threshold can only be determined if the experimental design allows for the reduction of nutrient concentrations. Rier and Stevenson (2006) tried to address this problem by using biological uptake of nutrients in re-circulating flumes as a means of reducing nutrient concentrations. A new methodology, developed by Bowes *et al.* (2007), used the addition of an iron sulphate solution to river water to reduce phosphorus concentration in streamside flumes. For the first time, this allowed reduced nutrient concentrations to be measured and sustained at near constant concentrations for the duration of a manipulation experiment.

The Centre for Ecology and Hydrology (CEH) has since developed portable in-stream flume mesocosms (an open water system) that are capable of being

transported and deployed in different rivers where nutrient concentrations can then be increased and, more importantly, decreased (see Section 2.1 for further details) (Bowes *et al.*, 2010a, Bowes *et al.*, 2012a). Unlike whole stream enrichment experiments, these in-stream flume mesocosms allow the ecological effects of multiple nutrient treatments to be studied simultaneously. The portability of the mesocosms, combined with minimal power requirements, allows experiments to take place at sites of specific scientific or environmental interest. They are, therefore, more flexible than other laboratory or streamside flume designs. Consequently, they were used as the primary method of investigation of nutrient limitation in this thesis.

1.11 Research questions

As stated in Section 1.2, the overarching question addressed in this thesis is "what is the phosphorus-limiting threshold for UK rivers?" Although general nutrient targets have previously been suggested in the UK (Mainstone and Parr, 2002, UKTAG, 2008, UKTAG, 2013a), river-specific knowledge-based, threshold values have never been given. Further research questions which this thesis aims to address are:

1. Is phosphorus the limiting nutrient in UK rivers?
2. Are periphyton communities able to adapt to reduced phosphorus concentrations?
3. Can other abiotic variables be manipulated to reduce periphyton biomass and improve ecological status?
4. Are limiting phosphorus concentrations the cause of stress and the collapse of annual phytoplankton blooms?

1.12 Thesis structure

Chapter Two (Methods) details the general methodology used in the flume experiments described in Chapters Three to Five. This chapter also provides details of the sampling and analytical techniques used throughout the flume experiments and for water quality analyses.

Chapter Three presents a flume experiment run on the River Lambourn, West Berkshire. This chalk stream, a site of special scientific interest, has very good water quality compared with other rivers in the south-east of England. As well as examining periphyton response to a range of nutrient concentrations, light intensity was simultaneously manipulated, allowing the effects of both ecological drivers and the interaction between the two on the phosphorus-limiting threshold to be investigated.

Chapter Four details the results of a flume experiment conducted on the River Rede, Northumberland. The River Rede has little anthropogenic impact and extremely low nutrient concentrations, providing a contrast to the more nutrient enriched river sites in the south-east of England.

Chapter Five examines whether or not periphyton communities can adapt to reduced ambient phosphorus concentrations, using the River Frome, Dorset as a case study. The river was the site of a similar nutrient limitation experiment using streamside flumes in 2005 (Bowes *et al.*, 2007), but has seen large improvements in its water quality (particularly reduced SRP concentration) since then. This experiment was designed to see if the reduction in phosphorus concentration resulted in periphyton limitation (as predicted by Bowes *et al.*, 2007), or whether the periphyton communities were able to adapt to their new nutrient regime.

Chapter Six is a synthesis of the results from the flume experiments on the River Lambourn, Rede and Frome and discusses the likely nature of nutrient limitation across the UK.

Chapter Seven details the use of a fast repetition rate fluorometer across the Thames catchment, to determine phytoplankton fluorescence yield and measure phytoplankton stress. These data, coupled with extensive water chemistry data, were used to determine the factors affecting and controlling the annual algal bloom.

Chapter Eight (Conclusion) discusses the implications of the thesis findings within the context of the research questions, and provides an overview of phosphorus-limiting thresholds in UK rivers. Suggestions for further work, as a result of the findings, are also provided.

Chapter 2: Methods

A number of methods used throughout this thesis were based upon common experimental design procedures and laboratory analyses. Consequently, the general methods used are described in this chapter, while experiment-specific details are given in the relevant chapters.

2.1 Flume mesocosms

In these studies, nutrients and iron salts were added to in-stream through-flow flume mesocosms in order to simultaneously increase and decrease phosphorus concentrations. Nitrate was also added to some flumes, to increase the bioavailable nitrogen concentration of the incoming river water. For each experiment, twelve or fifteen flume mesocosms were deployed on a straight section of river with minimal riparian shading. The sites were chosen so that they were accessible, but away from public view and had a suitable water depth. Suitable depth was defined as deep enough to allow the flumes to float but shallow enough for fieldworkers to operate safely.

Each structure consisted of three flumes which were each 5 m long and 0.25 m wide (Figure 2.1). The upstream end of each flume was fitted with adjustable gates to allow the velocity of the incoming river water to be controlled, allowing identical flow velocities to be produced across all the flumes. The water velocity in each flume at the start of each experiment was measured using a Valeport electromagnetic flow meter (model 801; Valeport Ltd., Totnes, UK). The flumes were constructed from polyvinyl chloride (PVC) sheeting and each set of three flumes was supported within an aluminium frame to prevent buckling against the force of the water and with changing temperature.

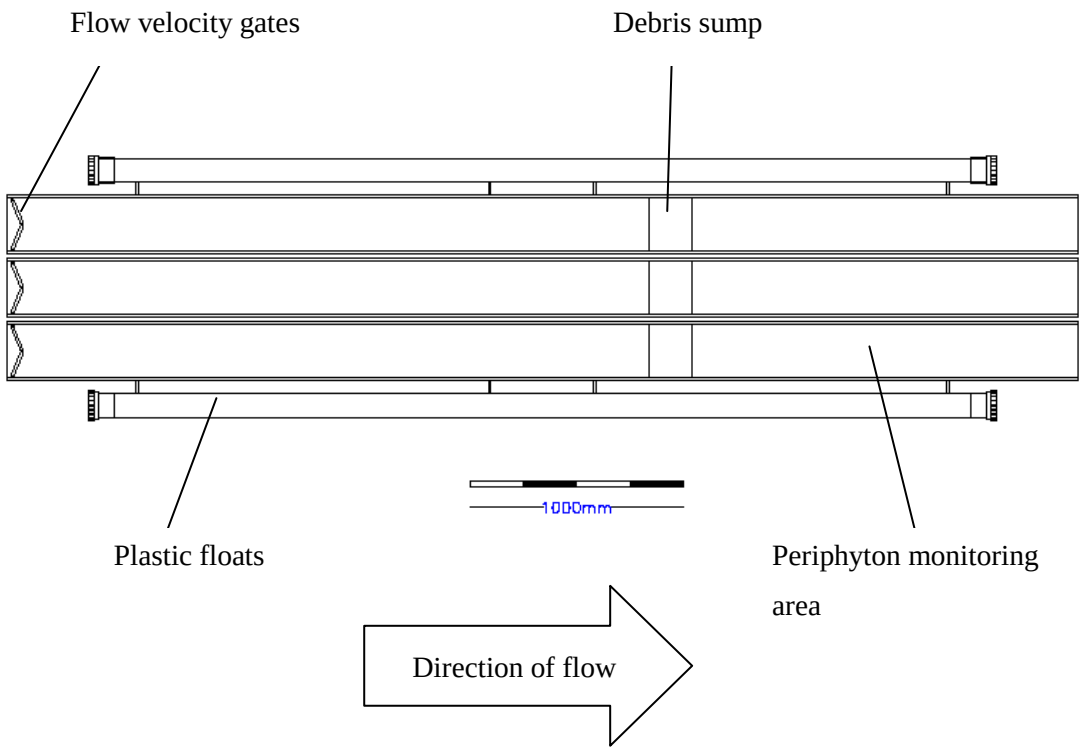


Figure 2.1: A scale drawing of the flume structure containing three individual flumes.

Sealed plastic cylindrical tubes were clipped to the sides of each set of three flumes enabling them to float at a constant depth of approximately 4 cm, irrespective of fluctuating river levels throughout the experiment. Because the flumes were maintained at a constant height within the water column, and not in contact with the river bed, grazing of periphyton by invertebrates was kept to a minimum. For three of the five experiments within this thesis, flumes were secured in place by positioning them over metal scaffolding poles that had been pile driven into the river bed (Figure 2.2). For the final two experiments (River Frome), water levels were too high to secure the flumes using the scaffolding poles, and so the poles were inserted into the river bank and the flumes secured with rope (Figure 2.3). Ropes were then manually adjusted and slackened as river levels dropped throughout the course of the experiments. A sump (25 x 25 cm with a depth of 60 cm) was located two-thirds of the way down each flume. The sump allowed any debris, such as leaves and twigs,

that had entered the flume to be deposited before reaching the periphyton monitoring area in the downstream section of the flumes (Figure 2.1), as this debris may disturb the accrued biofilm.

Figure 2.2: Photographs of flume deployments. (A) Two sets of flumes secured in place with scaffolding poles at the River Rede study site. (B) Two sets of flumes secured in place with rope at the River Frome study site. The blue arrow represents direction of river flow.

Temperature and light levels in the sets of flumes were measured hourly using HOBO pendant loggers (Onset Computer Corporation, Massachusetts, USA) for the duration of each experiment. In addition, one logger was placed in the main river channel to record water temperature and one placed on the river bank to record sunlight intensity at hourly intervals. Prior to placing in the flumes, loggers were calibrated in the laboratory to ensure each logger was reading the same temperature and light values. This was achieved by placing all loggers in a bowl of water and recording light and temperature values every 5 minutes for 2 hours. The precision of the HOBO's was ± 0.5 °C and ± 200 Lx. The light sensor on each logger was cleaned daily throughout the duration of the experiment in order to remove any accumulated periphyton. Random checks of water temperature within the flumes and main river channel were also made throughout the experiment using a digital thermometer.

The water depth within each flume (in centimetres) was measured and recorded during each experiment using a rule. The depths of each set of flumes were made the same prior to the start of each experiment, by adjusting the positions of the clips attaching the floats to the flumes.

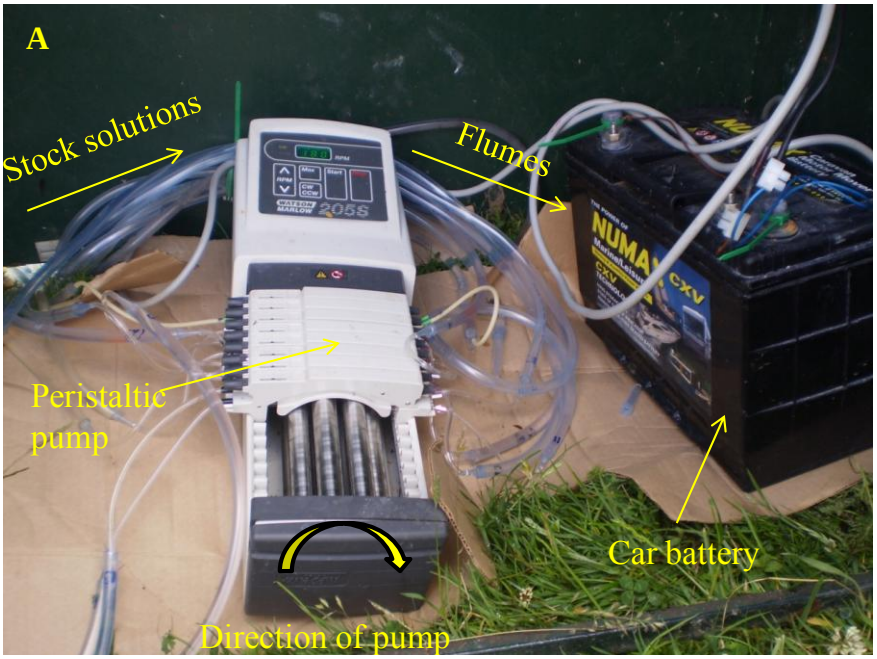
During each experiment, peristaltic pumps (model 205S; Watson Marlow, Falmouth, UK) (Figure 2.3A) delivered a continuous drip rate of nutrients to each flume from stock solutions. The stock solutions were made up from deionised water spiked with either dissolved potassium orthophosphate (KH_2PO_4), sodium nitrate ($NaNO_3$) or iron (II) sulphate ($FeSO_4$) / iron (III) chloride ($FeCl_3$) and mixed well, to ensure the salts were fully dissolved. The addition of iron allowed the ambient SRP concentration of the incoming river water to be reduced and follows the phosphorus-stripping methodology developed by Bowes *et al.* (2007). The added iron reacted with the dissolved phosphate ions present in the incoming river water, rapidly forming an insoluble, non-bioavailable precipitate ($Fe_3(PO_4)_2$) (Reynolds and Davies, 2001).

Once water chemistry was stable, all flumes were thoroughly scrubbed to remove any periphyton that had colonised during the setting up of the experiment. Any invertebrates that were found were also removed. Next, unglazed quarry tiles (approximate area 49 cm^2) were placed in the downstream section of each flume to

act as artificial substrate for periphyton growth. Prior to being placed in the flumes, the quarry tiles had been thoroughly scrubbed and washed with deionised water. Tiles were placed in each flume approximately 5 cm apart and positioned towards the middle to ensure they were not shaded by the sides of the flume. Orientation of artificial substrates has been shown to affect periphyton biomass and community composition (Murdock and Dodds, 2007). However in other studies, it was found to have no significant effect (Cattaneo and Amireault, 1992). To avoid doubt, the artificial tile substrates used in this thesis were all placed in the same orientation.

Aluminium foil was placed around the stock solution bottles in order to minimise light reaching the solution and to reflect heat, thereby minimising temperature increases, in an attempt to reduce biological growth from occurring in the stock solutions (Figure 2.3B).

Within each set of three flumes, there was always one control flume which had no nutrient additions and therefore received unmodified river water for the duration of the experiment. Having a control flume in each set of three flumes allowed the results from treatment flumes to be compared between the different sets of flumes.



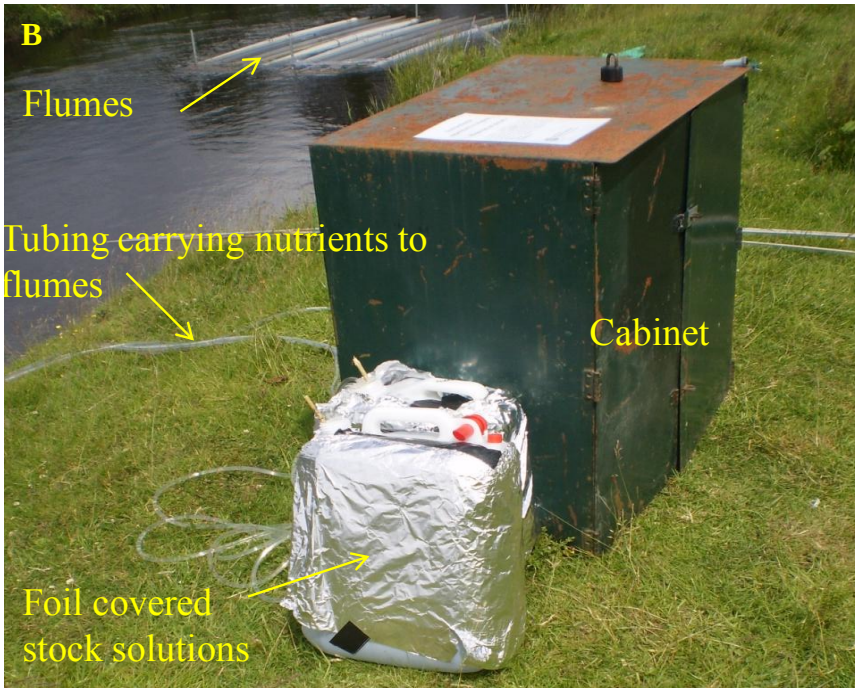


Figure 2.3: Photographs showing (A) the inside of the bank side cabinet with the peristaltic pump being powered by a car battery. (B) The bank side cabinet containing the peristaltic pump and aluminium foil around the stock solution bottle in order to reflect heat and prevent algal growth in the stock solutions.

The experiments ran for between 6 (River Frome) and 11 (River Lambourn) days. Experiments were terminated once significant quantities of periphyton had accrued on the tiles and sloughing appeared to be imminent in some flumes. The time it took to reach maximum periphyton biomass was affected by river temperature, sunlight intensity and duration, and the time of year the experiment took place.

For the duration of each experiment, water samples (20 ml) were taken approximately three times per day from the area immediately above the tiles in each flume. These were immediately filtered through sterile 0.45 µm cellulose nitrate membrane filters (WCN grade; Whatman Ltd., Maidstone, UK) and analysed within 20 minutes in the field for SRP concentration, using a portable spectrophotometer (model DR2800; Hach Lange, Düsseldorf, Germany) (details given in Section 2.4.3). The daily nitrate concentrations of the flumes were determined by colorimetry by the addition of 2, 6-dimethylphenol (LCK 339 cuvette test; Hach Lange, Düsseldorf, Germany). These in-field analyses informed the altering of nutrient drip rates and concentration of stock solutions in order to maintain stable nutrient concentrations in each flume throughout the experiment.

On the final day of each flume experiment, one tile from each flume was placed into a labelled zip-lock bag and frozen at -20 °C for analysis of phosphorus, nitrogen and carbon concentration of the accumulated periphyton biofilm. A further three tiles were frozen for later analysis of chlorophyll-*a* concentration, dry mass (DM) and ash free dry mass (AFDM). These analyses provide proxies for periphyton accrual rate. In addition to these final-day samples, tiles were also removed at regular intervals throughout the experiments to allow temporal changes to be investigated. One tile from each flume was removed on the final day of the experiment for later identification of diatom species. The tile was placed in a plastic tray and scrubbed clean using a toothbrush and deionised water. The resulting suspension was poured into a Sterilin tube and 1 ml of 40 % formaldehyde was added to preserve the sample. The diatom sample was refrigerated at 4 °C and kept in the dark.

2.2 Periphyton analysis

Three tiles from each flume were defrosted in the dark before being scrubbed with a toothbrush into a plastic tray. The tiles, toothbrush and storage bags were then rinsed thoroughly with deionised water to ensure all material had been removed. After shaking the tray gently to re-suspend any periphyton / sediment that had settled, the suspension was poured into a 500 ml Duran Bottle (Fisher Scientific, Loughborough, UK) and made up to 300 ml with deionised water.

2.2.1 Chlorophyll-*a* analysis

The 300 ml periphyton suspension samples were homogenised for 10 minutes by placing on a magnetic stirrer plate. A 40 ml sub-sample was taken and filtered through a 0.45 µm membrane glass fibre filter paper (GF/C grade; Whatman Ltd., Maidstone, UK) under vacuum. Filter papers were placed into individually labelled vials and 20 ml of 90 % (v/v) acetone was added. The vials were then placed in a dark refrigerator (4 °C) overnight to extract chlorophyll-*a*. The following morning, vials were removed from the refrigerator, inverted to mix the sample, and the filter papers were removed using tweezers. The samples were then left in the dark for one hour to allow any sediment to settle and to equilibrate to room temperature. Approximately 3 ml of liquid from each sample was transferred to a 1 cm cuvette using a glass Pasteur pipette. Between each sample, the pipette and cuvette were rinsed thoroughly using 90 % acetone to prevent cross-contamination. The cuvette was then wiped clean using lens tissue and the absorbance of each sample was measured at wavelengths of 630, 645, 665 and 750 nm (APHA., 2005) using a spectrophotometer (model DU520; Beckman Coulter, California, USA).

The chlorophyll-*a* concentration (µg cm^{-2}) for each sample was determined using Equation 2.1 below (APHA., 2005). To quantify the chlorophyll-*a* concentration per tile, the tile area in the sub-sample was calculated and applied to the chlorophyll-*a* equation.

$$\text{Chlorophyll–}a = \frac{\left(11.85 \times (OD665)\right) - \left(1.54 \times (OD645)\right) - \left(0.08 \times (OD630)\right) \times \text{volume of acetone (ml)}}{\text{tile area in sub-sample (cm}^{-2}\text{)}}$$

[Equation 2.1]

Where OD_{665} = absorbance$_{665}$ – absorbance $_{750}$

OD_{645} = absorbance $_{645}$ – absorbance $_{750}$

OD_{630} = absorbance $_{630}$ – absorbance $_{750}$

The absorbance measurement at 750 nm is a correction for turbidity so was subtracted from each of the absorbance measurements at 630, 645 and 665 nm to give the optical density (OD) before being entered into the equation (APHA., 2005).

2.2.2 Dry mass and ash free dry mass analysis

The methods described below were adapted from Steinaman and Lamberti (1996). From the 300 ml homogenised periphyton sample (prepared in Section 2.2), a second 40 ml sub-sample was taken. The sub-sample was filtered through an ashed, pre-weighed glass fibre filter paper (GF/C grade; Whatman Ltd., Maidstone, UK). The filter papers were dried at 105 °C to constant mass (approximately 15 hours) before being cooled in a desiccator and re-weighed. The dry mass (DM) (mg cm^{-2}) was then calculated according to Equation 2.2. As with chlorophyll-*a* concentration, the mass per tile was calculated by dividing the mass by the tile area within the sub-sample.

$$DM = \frac{\text{mass of filter paper + dry sample (mg) - mass of filter paper (mg)}}{\text{tile area in sub-sample (cm}^{-2}\text{)}}$$

[Equation 2.2]

Following this, the filter papers were placed in a muffle furnace (model AAF 1100; Carbolite Ltd., Hope, UK) and incinerated at 500 °C for two hours to volatilise all organic material. After cooling in a desiccator, filter papers were re-weighed to the nearest milligram and the ashed mass (AM) (mg cm^{-2}) was determined according to Equation 2.3.

$$AM = \frac{\text{mass of filter paper + ashed sample (mg) - mass of filter paper (mg)}}{\text{tile area in sub-sample (cm}^{-2}\text{)}}$$

[Equation 2.3]

The DM and AM were used to calculate the ash free dry mass (AFDM) (mg cm^{-2}) according to the Equation 2.4. AFDM is a useful metric for measuring biomass as it is the mass of all organic material (algae, bacteria, small fauna and detritus) in the sample in the absence of silt, sediments and other inorganic components (APHA., 2005).

$$AFDM = \text{dry mass (mg cm}^{-2}\text{)} - \text{ashed mass (mg cm}^{-2}\text{)}$$

[Equation 2.4]

2.2.3 Autotrophic index

The autotrophic index (AI) is the ratio of AFDM to chlorophyll-*a* concentration and is a means of determining the trophic nature of the periphyton community (Ameziane *et al.*, 2002, APHA., 2005). It was calculated by Equation 2.5.

$$AI = \frac{\text{AFDM (mg m}^{-2}\text{)}}{\text{Chlorophyll-}a\text{ concentration (mg m}^{-2}\text{)}}$$

[Equation 2.5]

In general, higher AI values are an indication of heterotrophic dominated communities. However, the index must be interpreted with caution as it is affected by non-viable organic matter (detritus) which can inflate the AFDM affecting the index (APHA., 2005).

2.2.4 Elemental stoichiometry

2.2.4.1 Nitrogen and carbon

For each flume, the frozen periphyton was removed from the tile using a scalpel and placed into individually labelled and weighed crucibles. These were then placed in a drying oven and dried at 105 °C for approximately 48 hours until constant mass was attained. After cooling, approximately 100 mg of each sample was removed and stored in a Sterilin tube for analysis of total carbon (TC) and total nitrogen (TN). TC and TN were analysed by high temperature catalytic oxidation using a Vario TOC Select system (Elementar, Hanau, Germany) (Figure 2.4). Samples were weighed into tinfoil cups and the exact sample weight recorded. The cups were then sealed and compressed to form tin capsules, before being placed in to the autosampler carousel (Figure 2.4). Prior to the samples being run, three conditioning samples were run and a calibration was completed using sucrose as the carbon standard (0, 5, 10 and 30 mg, absolute carbon content = 42.1 %) and potassium nitrate as the nitrogen standard (0, 2, 5, 10 and 15 mg, absolute nitrogen content = 13.9 %). The weight of each sample and standard was entered into the computer software to calculate the percentage of carbon and nitrogen.

The packed sample was dropped into the combustion tube filled with a copper oxide catalyst which was maintained at a temperature of 950 °C (Elementar, 2010). The combustion tube was enriched with oxygen so that organically bound carbon and nitrogen within the sample burned, releasing carbon dioxide and nitric oxide. The carrier gas flow then transported the gaseous combustion products into the separation unit where volatile halogen compounds were removed via adsorption to silver wool and water was removed by a condenser and by an absorption tube filled with magnesium perchlorate. An infrared detector measured the signal provided from the combustion products (Elementar, 2010). The concentrations of TC and TN were then quantified by peak area of the calibration curve constructed (Elementar, 2010).

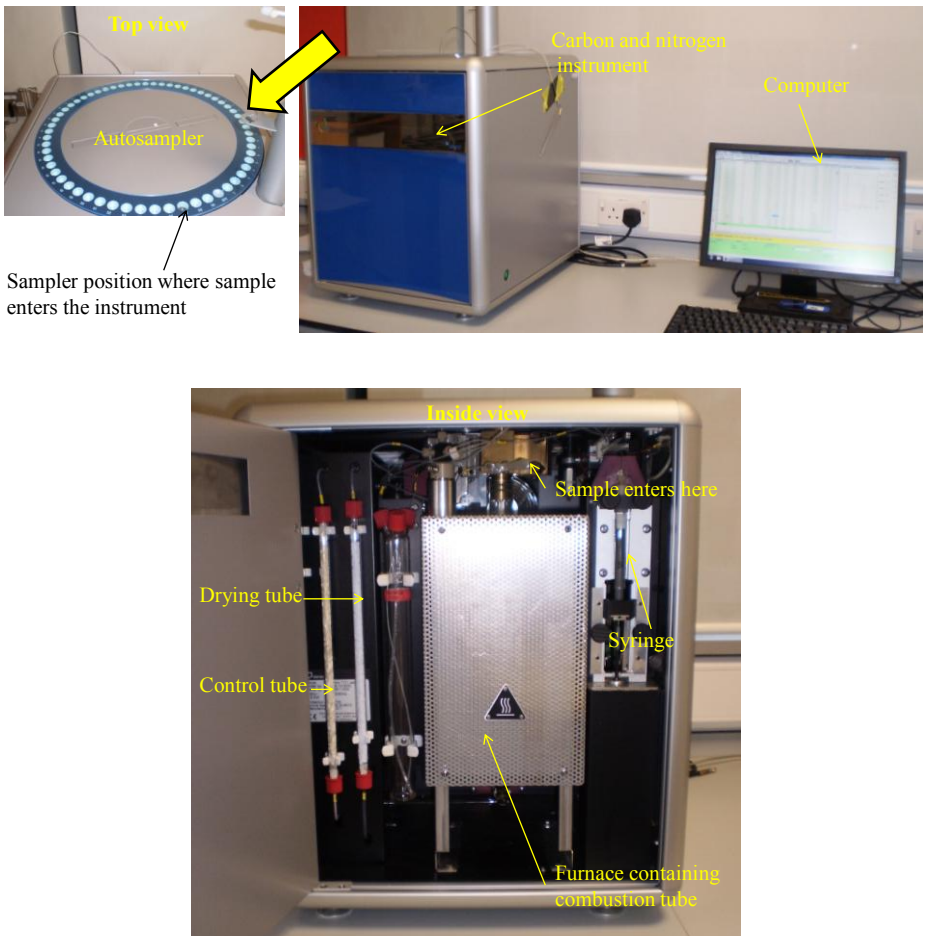


Figure 2.4: Annotated photographs of the Elementar carbon and nitrogen analyser.

2.2.4.2 Phosphorus

The remaining dried periphyton samples (after sub-samples for total carbon and total nitrogen were removed) were incinerated at 550 °C for three hours in a muffle furnace. The resulting ash was ground to a homogenous powder using a mortar and pestle before triplicate subsamples of approximately 3 ± 0.1 mg were taken.

Next, 60 ml of ultrapure water was added to each sample and thoroughly mixed. A 20 ml aliquot of this was taken and transferred to a 60 ml polyethylene bottle. To this, 1 ml of 1.01 N sulphuric acid (H_2SO_4) and 0.15 ± 0.1 g of potassium persulphate ($K_2S_2O_8$) were added and mixed gently by inverting. Reagents and chemicals used in all analyses were of analytical grade. Alongside each set of samples, a set of standards of known phosphorus concentrations and accredited

external reference phosphorus standards (LGC Aquacheck, Lancashire, UK) were measured.

After the sulphuric acid and potassium persulphate addition, samples were autoclaved by heating to 121 °C for 45 minutes at a pressure of 103.4 kPa. Autoclaving digested and removed organic matter, thereby releasing organically bound phosphorus which subsequently underwent hydrolysis to convert organic phosphorus to soluble, inorganic phosphorus (Mackereth *et al.,* 1989). Where samples were likely to be off-scale due to high phosphorus concentrations (> 700 µg l^{-1}), they were diluted with ultrapure water prior to the addition of sulphuric acid and potassium persulphate.

While the autoclave process was occurring, an acid-antimony molybdate reagent was made by dissolving 0.57 g of antimony potassium tartrate into approximately 500 ml of ultrapure water. Once dissolved, 45 ml of concentrated sulphuric acid were added while stirring using a magnetic stirrer and the solution left to cool. Meanwhile, a second solution consisting of 8.52 g of ammonium molybdate dissolved in 400 ml of ultrapure water was made. Once cool, the two solutions were mixed together and diluted to 1 l with ultrapure water in a volumetric flask, to produce a concentrated reagent mix. This reagent mix was stored at room temperature in an amber bottle and was stable for several months. Immediately before analysis, 50 ml of the reagent mix was measured in a volumetric flask and 0.31 g of ascorbic acid was dissolved into it to produce the 'working' reagent.

After cooling the samples to room temperature, 1 ml of the 'working' reagent consisting of acid antinomy molybdate and ascorbic acid was added. Twelve minutes then elapsed allowing the blue colour to develop (Figure 2.5A). TP concentration was then determined using the molybdate – blue reaction by measuring the absorbance at a wavelength of 880 nm on a spectrophotometer (Varian; California, USA) (Eisenreich *et al.*, 1975) (Figure 2.5B).

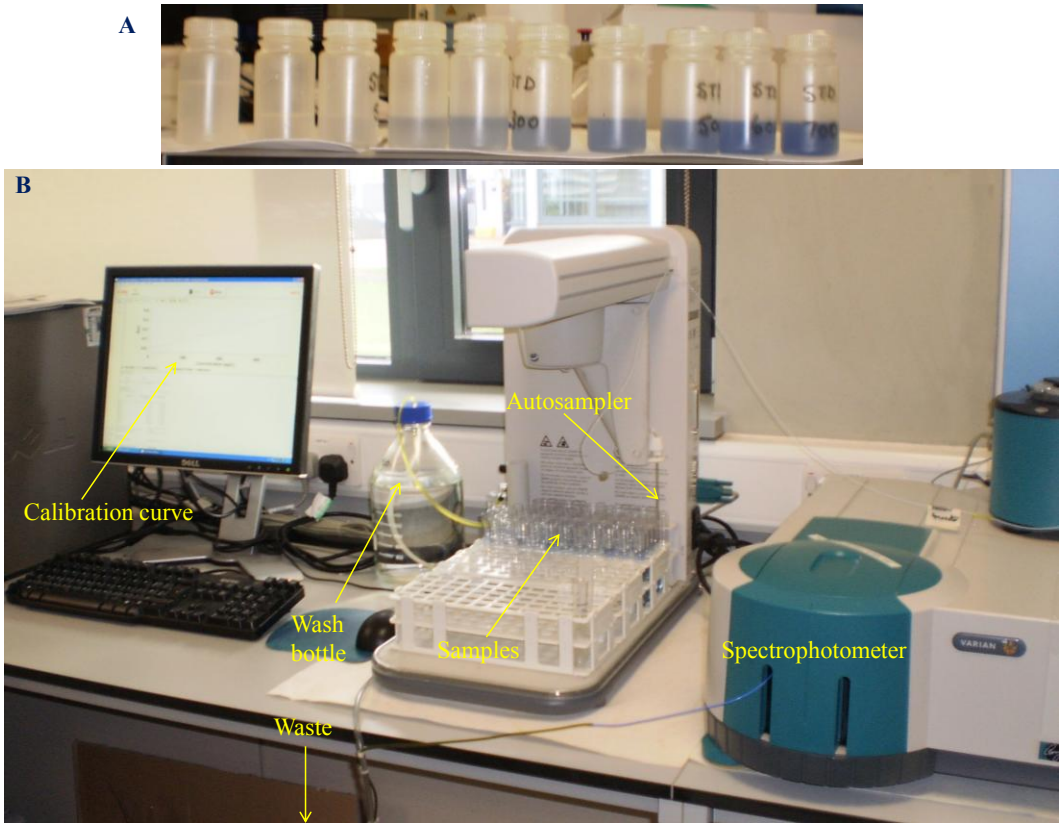


Figure 2.5: Annotated photograph of Varian total phosphorus analyser, showing (A) the blue colour developing as the molybdate reaction proceeds and (B) the total phosphorus / total dissolved phosphorus system.

A calibration curve was produced based on the standard solutions ranging from 0 to 700 µg-P l^{-1} (Figure 2.5A). It was from a linear regression of this calibration curve that sample TP concentrations were quantified giving phosphorus concentrations in units of micrograms per litre (µg l^{-1}). To convert this to micrograms per milligram of AFDM (µg mg $AFDM^{-1}$) (Fanta *et al.*, 2010), the value was divided by 1000 and multiplied by 60 to give the TP concentration within each sample, and then divided by the AFDM of the sample used for the analysis. To allow elemental stoichiometry to be determined and calculate nutrient ratios within periphyton biofilms, phosphorus data was converted to percentage phosphorus within the biofilm according to Equation 2.6.

$$\% \text{ P} = \frac{\text{TP in sample (µg)}}{\text{(AFDM (mg) x 1000)}} \text{ x } 100$$

[Equation 2.6]

2.2.4.3 Nutrient ratios

In order to be able to calculate nutrient ratios, see how these change with nutrient manipulations and compare these to the Redfield ratio of C: N: P equals 106: 16: 1 (Redfield, 1958), the percentage of each nutrient was first converted to grams per gram of sediment and then to moles of each nutrient per gram of sediment according to Equation 2.7 and Equation 2.8.

$$\text{grams of nutrient per gram of sediment} = \frac{\text{percentage of nutrient}}{100}$$

[Equation 2.7]

$$\text{moles of nutrient per gram of sediment} = \frac{\text{Equation 2.7}}{\text{atomic mass of the nutrient}}$$

[Equation 2.8]

From the molar ratios, it was possible to calculate the C: N: P ratio by dividing the moles of carbon and nitrogen by the moles of phosphorus within the periphyton biofilm.

2.2.5 Diatom slide preparation

Due to the speed at which diatom communities respond to environmental change, it was possible to examine changes in community composition over the (relatively) short time period over which the experiment ran. Microscopic slides for the analysis of diatoms within the periphyton community for each flume were prepared. Samples (scrubbed material preserved in 40 % formalin) were removed from the refrigerator and left to equilibrate to room temperature. They were then vigorously shaken to

ensure they were thoroughly mixed and *ca.* 5 ml was placed into a Pyrex beaker and cleaned using the 'hot peroxide method' (Kelly *et al.*, 2001).

Approximately 40 ml of 30 % hydrogen peroxide solution (H_2O_2) was added to each sample and gently heated to 90 ± 5 °C for approximately three hours. The reaction was complete when all organic matter had been removed, as indicated by the cessation of bubbling and the solution becoming colourless. These sample / peroxide mixtures were removed from the heat and allowed to cool before a few drops of 10 % hydrochloric acid (HCl) were added to remove any carbonates. Samples were then transferred to individually labelled centrifuge tubes which were filled to 1 cm below the rim with ultrapure water. Samples were centrifuged at a speed of 3000 rpm for 5 minutes, after which the supernatant was discarded before the centrifuge tube was refilled to 1 cm below the rim with ultrapure water. This washing process occurred three times in total to ensure all traces of hydrogen peroxide had been removed.

The cleaned sample material was placed on to cover slips (two per sample) and mounted onto slides. The vials were shaken to thoroughly mix before a drop of the suspension was transferred to a cover slip using a Pasteur pipette. The excess liquid was allowed to evaporate at room temperature in a clean, dust-free environment. Prior to permanent mounting, the density of diatoms on the cover slip was checked under a medium power objective (400 x) so that there was a maximum of 30 valves per field of view. To fix the cover slips and create permanent slides, Naphrax (refractive index= 1.74) (Brunel Microscopes Ltd., Chippenham, UK) was used. Slides were gently heated using a hot plate and a drop of Naphrax placed onto them with heating continuing until the Naphrax began to bubble and spread. The cover slip was then placed face down onto this and gentle pressure was applied with forceps to ensure all air bubbles were removed. Slides were then labelled with the sample collection date, site and flume number and stored in a clean, dust-free environment until diatom identification could take place.

2.2.6 Diatom identification and the trophic diatom index

At least 300 undamaged valves were identified and counted for each sample using a DMLB2 microscope (Leica Microsystems Ltd., Milton Keynes, UK). The

microscope was set at 100 x oil immersion under phase contrast. Identification of diatom assemblages was carried out following the diatom key developed by Kelly *et al.* (2005). All taxa were identified to the highest possible resolution, usually species or variety. Where experiments had a shaded treatment, only diatoms in unshaded samples were identified.

Species lists were used to calculate the trophic diatom index (TDI) (Equation 2.10), a key index used to assess ecological status for the WFD. The TDI indicates floristic change as a result of the effects of nutrients (generally phosphorus) (Kelly *et al.*, 2001). The index has a scale of 0 to 100 with higher values representing higher levels of nutrients and degrading water quality (Kelly *et al.*, 2001). It was calculated from the weighted mean sensitivity (WMS) of all taxa present in the sample (Equation 2.9).

$$WMS = \frac{\sum as}{\sum a}$$

[Equation 2.9]

Where a = abundance - number of a particular species in a sample / total number of species

s = nutrient sensitivity of a particular species (Kelly *et al.*, 2008)

$$TDI = (WMS \times 25) - 25$$

[Equation 2.10]

Ecological status at each flume site and how the addition and removal of nutrients affected this was determined based on the TDI calculated in Equation 2.10 and the expected TDI (eTDI) under reference conditions (Equation 2.11) (UKTAG, 2013b). Annual mean alkalinity at each site was determined using methods described in Section 2.4.9. Where alkalinity was below 5 or above 250 mg l^{-1} of calcium carbonate, values were set to 5 and 250 respectively. From the eTDI an ecological quality ratio (EQR) was calculated (Equation 2.12) which corresponded to classes relating to ecological status (Table 2.1). Where the calculated EQR was greater than 1.25, the value was set to 1.25.

$$eTDI = 9.933 \times \text{Exp}\,(\log_{10}(\text{alkalinity}) \times 0.81)$$

[Equation 2.11]

$$EQR = \frac{(100\text{-observed value of river TDI})}{(100\text{-expected value of river TDI})}$$

[Equation 2.12]

Table 2.1: Ecological quality ratio scores and corresponding classes of ecological status (UKTAG, 2013b).

Ecological quality ratio (EQR)	Ecological status class
> 1	High
0.76 – 1.00	Good
0.51 – 0.75	Moderate
0.26 – 0.50	Poor
0 – 0.25	Bad

2.3 Data analysis

Statistical analyses were based on mean values of three tiles with the standard error being calculated and displayed on graphs. Histograms and probability plots were used to ensure data were normally distributed and Bartlett's test for homogeneity of variances was used to test the data for heteroscedasticity. Where necessary, data were transformed prior to further analysis using a Box-Cox transformation and re-analysed for normality and heteroscedasticity (Quinn and Keough, 2002).

Once data met pre-requisites, one-way analysis of variance (ANOVA) tests were run on mean control values for chlorophyll-*a* concentration, AFDM and periphyton elemental concentration in each experiment. This ensured rate of periphyton accrual was similar when all conditions (apart from nutrient treatment) were the same. Where this indicated a significant difference ($p < 0.05$), data were normalised to the control in each set of three flumes. Normalisation was undertaken by taking the result in the treatment flume and dividing it by the result in the control flume within each set of three flumes so that for each variable, the control flume was equal to one (Quinn and Keough, 2002).

Once differences in controls had been assessed, differences in mean chlorophyll-*a* concentration, AFDM and periphyton elemental concentration were tested in the River Lambourn and River Frome experiment using one-way ANOVA. Where significant differences were found, post-hoc comparisons (Tukey's HSD test) were undertaken in order to determine where the significant differences lay. In addition to running statistical tests on variables associated with periphyton accrual, tests were also run on physical data such as light and temperature, which were recorded for the different sets of flumes (see Section 2.1).

Where a linear relationship was expected (the River Rede), the relationship was tested using model II regression. Model II regression was used (over model I regression) because both the x and y parameters (i.e. average soluble reactive phosphorus concentration and chlorophyll-*a* concentration, AFDM or periphyton elemental content) were dependent variables (i.e. both were measured and both include a source of error). In this situation, model I regression underestimates the slope of the linear relationship (Legendre, 2001). Based on the assumptions given by Legendre (2001), the specific regression test used was ranged major axis regression. All statistical analyses were run in Minitab version 15 with model II regression being run within a specific Fortran program (Legendre, 2001).

2.4 Water quality analysis

2.4.1 Longitudinal surveys

The use of longitudinal river surveys during the individual studies and throughout the following year meant that flume experiment data and conclusions drawn from a particular site at a particular point in time could be related to the catchment as a whole. For each survey, water samples were collected along the main stem of the study river and its major tributaries.

A bulk water sample was collected from the main flow of the river at each site. Two unfiltered 60 ml aliquots were taken from this bulk sample, using a syringe, for metals and TP analysis. A further three 60 ml aliquots were taken and filtered through a sterile 0.45 µm cellulose nitrate membrane filter paper (WCN grade; Whatman, Maidstone, UK) for dissolved metals, TDP and major anions and cation

analysis (silicon, dissolved organic carbon, total dissolved nitrogen, nitrite, nitrate and ammonia concentration). To reduce the risk of contamination between sampling sites, the filter paper was changed at each site and the syringe rinsed with river water prior to the sample being taken.

Samples were stored in the dark at 4 °C and returned to the laboratory as soon as possible for analysis in order to minimise sample degradation and any biological or chemical reactions occurring. Due to its instability (Haygarth *et al.*, 1995, House and Warwick, 1998) SRP was measured in the field (see Section 2.4.2). For this, a further 20 ml aliquot of water was filtered and stored in a Sterilin tube. Two 500 ml unfiltered samples were collected for analysis of suspended sediment and chlorophyll-*a* concentration. Finally, a brown glass bottle was filled to the top with unfiltered river water for pH and alkalinity analysis. Filling the bottle to the top minimised error associated with ion exchange with atmospheric gases.

2.4.2 Soluble reactive phosphorus analysis

SRP concentration was measured in the field using a portable spectrophotometer (model DR2800; Hach Lange, Düsseldorf, Germany) following the method of Murphy and Riley (1962). The phosphate present in the samples reacted with acid-molybdate to form molybdo-phosphoric acid. This complex was then reduced by the addition of ascorbic acid to form a blue complex. The intensity of this was measured spectrophotometrically and corresponded to the SRP concentration (Mackereth *et al.*, 1989).

The 20 ml aliquot of filtered river water was inverted (to mix it) before 1 ml of a colour reagent (R1) and 1 ml of a reducing reagent (R2) were added using a pipette (Murphy and Riley, 1962, Mackereth *et al.*, 1989). A batch quantity of 500 ml of each reagent was made in the laboratory prior to analysis in the field. To make R1, 60 ml of concentrated sulphuric acid were added to approximately 160 ml of deionised water while stirring. In a second beaker, 0.24 g of antimony potassium tartrate and 11.1 g of sodium molybdate dihydrate were dissolved in 200 ml of deionised water. Once fully dissolved, this salt solution was added to the acid solution and made up to 500 ml with deionised water. The mixed solution was then

decanted into a plastic bottle and stored in a sealed bag in a refrigerator. To make R2, 50 g of ascorbic acid was fully dissolved in 300 ml of deionised water. R2 was made up to 500 ml with deionised water, before being transferred to a dark plastic bottle. The solution was stored in a sealed plastic bag in a refrigerator.

Once the reagents were added, the samples were mixed and left to stand for 12 minutes to allow the colour to develop. The sample was then transferred to a rinsed cuvette and the absorbance measured at a wavelength of 880 nm. Reagents were simultaneously added to a range of phosphate standards with concentrations of 0, 25, 50 and 100 μg l^{-1}. These were plotted against the absorbance at 880 nm before a linear regression was undertaken to quantify the SRP concentration of the samples.

2.4.3 Total phosphorus and total dissolved phosphorus analysis

TP and TDP analysis of water samples followed a similar method to that described in Section 2.2.4.2 for biofilm internal phosphorus concentration. The 60 ml bottles of unfiltered (TP) and filtered (TDP) river water were vigorously shaken to mix any sediment that may have settled since collection. A 20 ml aliquot of the shaken sample was then transferred to a 60 ml polyethylene bottle and 1 ml of 1.01 N sulphuric acid and 0.15 ± 1 g of potassium persulphate were added. Samples were autoclaved at 121 °C for 45 minutes, 1 ml of working reagent added and after allowing time for the colour to develop, absorbance measured at a wavelength of 880 nm (Figure 2.5) (Eisenreich *et al.*, 1975).

2.4.4 Ammonia analysis

Ammonia analysis was undertaken using an AutoAnalyser Three system (AA3; Seal Analytical, Fareham, UK) to determine concentrations colorimetrically (Figure 2.6). Three reagents were used; salicylate, dichloroisocyanuric acid (DCI) and complexing reagent. The salicylate was made up weekly by dissolving 30 g of sodium salicylate in 60 ml of deionised water and diluting to 100 ml. Due to its sensitivity to light, this was stored in a dark brown glass bottle. The DCI was made fresh for each analysis. To do this, 3.5 g of sodium hydroxide were added to 80 ml of deionised water. Once this had completely dissolved, 0.2 g of DCI was added. The solution was diluted to

100 ml with deionised water and mixed thoroughly. The complexing agent was stable for two weeks and made by firstly dissolving 15 g of ethylenediaminetetraacetic acid (EDTA) in 400 ml of deionised water. To this, 60 g of tri-sodium citrate dehydrate and 0.25 g of sodium nitroprusside were added and allowed to dissolve. The solution was diluted to 500 ml with deionised water before 1.5 ml of brij-35 (30 % solution) was added to act as a wetting agent.

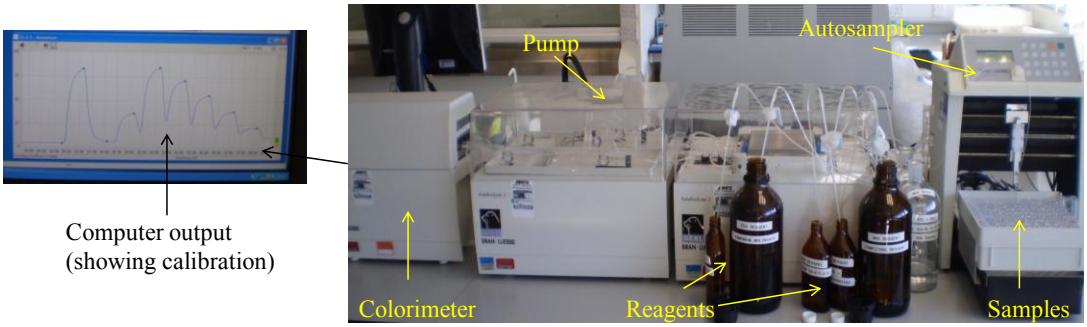


Figure 2.6: Annotated photograph showing the AA3 system used to measure ammonia concentrations.

The addition of sodium hydroxide to the DCI reagent ensured that the high pH necessary for the reaction to proceed was achieved and maintained (van Staden and Taljaard, 1997). Due to the high pH, metal ions would ordinarily precipitate out of solution forming insoluble hydroxides. Precipitation was avoided through the addition of the complexing reagent (Krom, 1980). Sodium nitroprusside was added to the complexing reagent as a catalyst allowing the reaction to proceed at room temperature. The reagents reacted together to form an intensely coloured indophenol dye, the absorbance of which was measured at a wavelength of 635 nm (Krom, 1980). As with measurements of phosphorus, the absorbance of a set of standards ranging from 0 to 0.2 mg l^{-1} of ammonia were measured at 635 nm and the regression from this calibration used to quantify sample values.

2.4.5 Soluble reactive silicon analysis

Soluble reactive silicon concentrations within water samples were analysed colorimetrically on a separate AA3 system. A set of standards with known dissolved silicon concentrations, ranging from 0 to 5 mg l^{-1}, were made up from a stock standard of Spectrosol silicon solution (concentration - 1000 mg l^{-1}). These were analysed at the beginning of each sample run with a subsequent linear regression from the absorbance of the standards being used to quantify silicon concentration within each sample. The precision of this calibration was validated with a check standard and an accredited external silicon reference standard (LGC Aquacheck, Lancashire, UK). Samples, reagents and standards were stored in polyethylene bottles, as storage in borosilicate glass has been shown to lead to higher blank values during calibrations (Fanning and Pilson, 1973).

In an acidic solution, silicon reacted with molybdate forming yellow molybdosilicic acids. These were then reduced by the addition of a reducing agent made up from oxalic acid and ascorbic acid to form silicomolybdenum blue compounds. The absorbance of this was measured at 810 nm and was proportional to silicon concentration (Mullin and Riley, 1955). The molybdate reagent (stable for two weeks) was made by dissolving 3.41 g of ammonium molybdate in 400 ml of 0.06 N sulphuric acid. This was then made up to 500 ml using 0.06 N sulphuric acid. Oxalic acid was made up by dissolving 59.3 g of oxalic acid in 800 ml of deionised water. To this, 1 g of SDS was added and dissolved. The solution was then diluted to 1 litre and mixed thoroughly before being stored in an amber plastic container. Finally, the ascorbic acid reagent was made up from 1.05 g of ascorbic acid dissolved in 200 ml of deionised water. To this, 2.4 ml of acetone were added and the solution was diluted to 250 ml with deionised water and stored in a polyethylene container. The ascorbic acid solution was stable for one week.

2.4.6 Metal analysis

Filtered and unfiltered water samples were analysed for metal concentration using inductively coupled plasma optical emissions spectroscopy (ICP-OES) (Perkin Elmer, Massachusetts, USA). Once returned to the laboratory, samples were acidified with 1 % (v/v) nitric acid to reduce adsorption loss and stored in a

refrigerator (4 °C). The specific metals measured were sodium, potassium, calcium, magnesium, boron, iron, manganese, zinc, copper and aluminium. ICP-OES has a number of advantages for metal analysis including; the ability to rapidly and simultaneously analyse a number of elements, a low detection limit, high precision and a wide linear dynamic range (Olesik, 1991). For quality control purposes, an accredited external reference standard (LGC Aquacheck, Lancashire, UK) and standard solutions of known concentrations were run alongside each set of samples.

Samples were removed from the refrigerator and placed in small test tubes in the autosampler. The sample was first passed through a nebulisation chamber that converted it into an aerosol, a fine mist consisting of small droplets. The resulting aerosol was then transported in the gas stream and passed through a spray chamber, which removed large droplets from the aerosol, draining them to a waste container. Smaller droplets of 10 µm (approximately 1 – 5 % of the sample that passed through the nebuliser) were then injected into the plasma. The plasma was maintained by an argon gas stream and was contained in the centre of the torch with a copper coil wound around it. Radiofrequency was applied to this to create a magnetic field which accelerated argon atoms. Argon was swirled through the torch and after application of a spark; electrons were removed from argon atoms to form argon ions with increased energy. This increased energy led to a chain reaction forming an inductively coupled plasma (Boss and Fredeen, 1997).

Once samples were in the plasma, high energy due to radiation and particle collision meant atoms and ions within the sample were desolvated, vaporised, dissociated, atomised, ionised and excited. Upon passing through and out of the plasma, atomic and ionic species relaxed, returning to their ground (stable) state. As this occurred, they released radiation as photons (Figure 2.7). The wavelengths of these photons were specific to each element (i.e. each element had its own unique emissions spectrum). Within the optical chamber it was, therefore, possible to measure the light intensity at each wavelength using a spectrophotometer (Boss and Fredeen, 1997).

The intensity of the emission was related to the concentration of the metal within the sample (Boss and Fredeen, 1997). The use of standard solutions of known concentrations allowed calibration curves to be constructed by plotting intensity

against concentration. Measured intensities for each element were then compared to these in order to quantify the metal concentration within the sample.

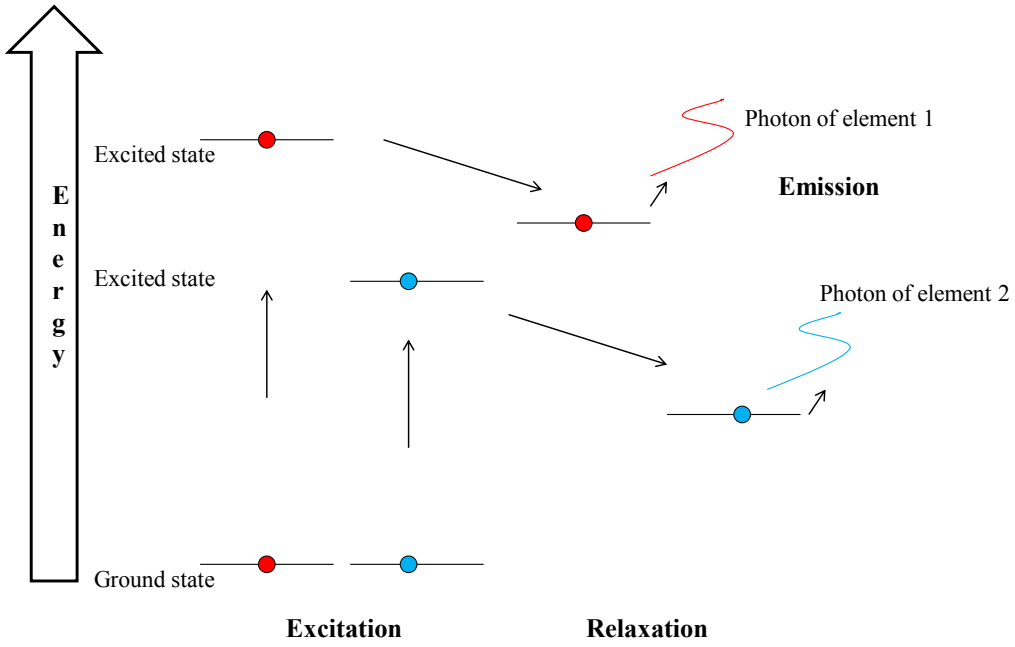


Figure 2.7: Schematic diagram of element excitation and photon emission through an inductively coupled plasma (ICP).

2.4.7 Ion analysis

A Dionex system (DX500; Thermo Scientific, Sunnyvale, USA) was used to measure concentrations of fluoride, chloride, bromide, sulphate, nitrite and nitrate by ion chromatography (Figure 2.8). The advantages of this process include a high sensitivity as indicated by the very low detection limit (within the parts per billion range), selectivity, simultaneous detection of ions, stability of the separator columns and speed of analysis, with each sample taking just six minutes to analyse (Weiss, 1995).

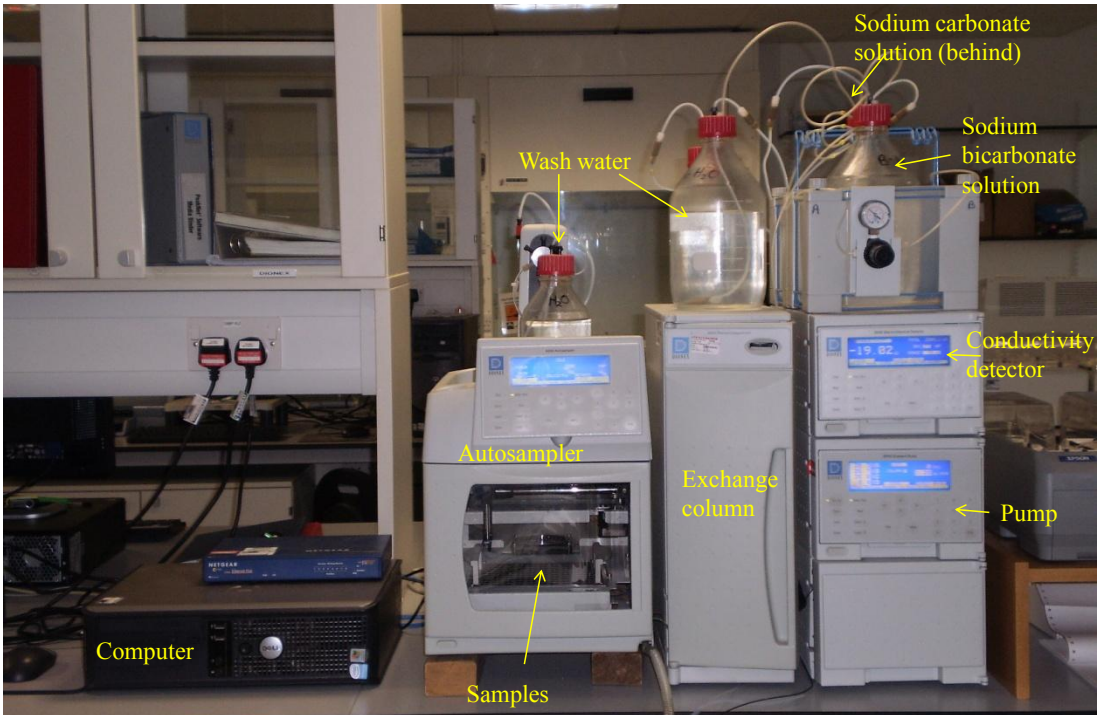


Figure 2.8: Annotated photograph showing the Dionex system.

The system was based on a simple process whereby an anion exchange resin was used to separate sample anions. A solution of sodium carbonate and sodium bicarbonate, known as the eluent, was pumped through the ion exchange system in order to replace all exchangeable ions in the system thus providing a constant signal from the conductivity detector (Haddad and Jackson, 1990). The eluent was made up by weighing 2.865 g of sodium bicarbonate and 3.816 g of sodium carbonate and dissolving this in 4 l of ultrapure water and was replaced with a fresh batch every two weeks. The eluent was sparged with nitrogen gas in order to create an anoxic environment within the exchange resin, thereby reducing interference from oxygen ions.

After the eluent had passed through the column, a small volume of sample was injected from the autosampler. Once injected, ions within the sample were exchanged with the same number of ions in the eluent. This maintained electroneutrality and resulted in a decrease in eluent ion concentration which created a peak (for each ion). Due to continuous eluent injection, the peak moved down the column. Different ions within the sample had different affinities for the resin exchange sites (different retention time) (Haddad and Jackson, 1990), meaning that

as samples moved down the column, the ions moved at different rates and the peaks became separated. The sample stream passed through the conductivity detector which measured the individual decreases in eluent ion concentration for each ion (peak area), the magnitude of which was directly proportional to the concentration of that ion in the sample (Weiss, 1995).

Alongside the samples in each run, five standard solutions of known concentrations, a check standard and an accredited external reference standard were run. A calibration curve was constructed from decrease in eluent concentration values for the standard solutions in order to quantify concentrations within the samples. The validity of this was checked using the result from the check standard and the accredited external reference standard (LGC Aquacheck, Lancashire, UK) (Weiss, 1995).

2.4.8 Carbon and nitrogen analysis

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were analysed by high temperature catalytic oxidation using a Vario TOC Select system (Elementar, Hanau, Germany) (Figure 2.4). DOC and TDN are operationally defined as the carbon and nitrogen fraction remaining in the sample after filtration through a 0.45 μm filter. River water samples were placed in individual vials within the autosampler. A 100 μl sample was injected into the combustion tube filled with a platinum catalyst which was maintained at a temperature of 850 °C (Elementar, 2010). The analysis then proceeded as described in Section 2.2.4.1. The concentrations of dissolved organic carbon and total dissolved nitrogen were then quantified by peak area analysis of a calibration curve constructed using standard solutions of potassium hydrogen phthalate (carbon compound) and sodium nitrate (nitrogen compound) (Elementar, 2010).

2.4.9 Alkalinity analysis

The alkalinity of each sample, a measure of water's capacity to neutralise acid, was measured using a double end-point titration (USEPA, 2012). From the glass bottle of unfiltered river water, 100 ml was measured and placed in a beaker with a magnetic

stirrer. Stirring the sample during the titration increased precision by minimising exchange of carbon dioxide with the air (Mackereth *et al.*, 1989).

The initial pH of the sample was recorded using a calibrated pH meter. Sulphuric acid (0.5 N) was added in increments of 50 µl until the pH was reduced to approximately 4.0. At this pH, hydroxide, carbonate and bicarbonate ions present in the sample have all been neutralised (USEPA, 2012). The volume of acid added and the exact pH reached were recorded. More acid was added to further reduce the pH to approximately 3.0. Below pH 4, there was a direct relationship between change in pH and the amount of acid added. The total volume of acid needed to reach this and the final pH were then recorded. The alkalinity of the sample was then back-calculated according to the Equations 2.13 to 2.15. Alkalinity was converted to units of mg l^{-1} of calcium carbonate (for use in TDI calculations – Section 2.2.6) by dividing the microequivalents per litre by 20 (20 µeq l^{-1} = 1 mg l^{-1} of calcium carbonate).

$$\text{Calculated titrant strength (eq } l^{-1}) = \frac{\left((V_0+V_{a4})\times 10^{-pH4}\right) - \left((V_0+V_{a3})\times 10^{-pH3}\right)}{(V_{a4}-V_{a3})}$$

[Equation 2.13]

$$\text{Intercept} = \left((V_0+V_{a4})\times 10^{-pH4}\right) - (\text{calculated titrant strength} \times V_{a4})$$

[Equation 2.14]

$$\text{Alkalinity (µeq } l^{-1}) = \frac{\left({}^{\text{intercept}}/_{\text{calculated titrant strength}}\right) \times 10^{-6} \times \text{acid stength}}{V_0}$$

[Equation 2.15]

Where: V_o = volume of solution to be titrated

Va_4 = volume of added titrate to reach *ca.* pH 4

pH4 = value of pH 4 recorded

Va_3 = volume of added titrate to reach *ca.* pH 3

pH3 = value of pH 3 recorded.

2.4.10 Chlorophyll-*a* analysis

Unfiltered bulk water samples (500 ml) were taken from the main flow of the river. The bottle was weighed (to the nearest gram) before being vacuum filtered through a GF/C grade filter paper (Whatman Ltd.; Maidstone, UK). The filtrate was discarded and filter papers placed in individual vials with 10 ml of 90 % (v/v) acetone for overnight extraction in a refrigerator. A glass rod was used to ensure the filter papers were fully submerged. The glass rod was cleaned between samples to minimise cross-contamination. Chlorophyll-*a* analysis then proceeded as described in (Section 2.2.1), with the absorbance being divided by volume in filtrate (rather than tile area in sub-sample as in Equation 2.1) to give chlorophyll-*a* concentration in micrograms per litre ($\mu g\ l^{-1}$) (APHA., 2005).

2.4.11 Suspended sediment analysis

Bulk water samples (500 ml) were weighed (to the nearest gram) before being vacuum filtered through a pre-weighed, dried, labelled GF/C grade filter paper. The empty bottle was rinsed with deionised water to ensure all remaining sediment was filtered and then re-weighed to determine the volume of water filtered (Beschta, 1996). To prevent cross-contamination, filtering equipment was washed with ultrapure water between each sample. The filtrate was discarded and filter papers were carefully removed and oven dried at a temperature of 105 °C overnight. After cooling to room temperature in a desiccator, samples were re-weighed to the nearest milligram using an analytical balance and the suspended sediment concentration calculated according to the Equation 2.16 (Beschta, 1996):

$$\text{Suspended sediment (mg l}^{-1}) = \frac{\text{mass of filter paper+sediment (mg)- mass of filter paper (mg)}}{\text{volume of filtrate (l)}}$$

[Equation 2.16]

Chapter 3: Light and nutrient limitation in the River Lambourn, West Berkshire.

3.1 Introduction

Phosphorus is widely believed to be one of the main controls of primary productivity (Chapter One). Light intensity is also an important factor in controlling excessive biofilm growth (Hill, 1996 and Section 1.8.1) as light is essential for carbon uptake in the process of photosynthesis and is the primary energy source for periphyton. Shading by riparian tree canopies has been shown to reduce photosynthetically active radiation (PAR) reaching stream water surfaces by up to 95 % (Hill, 1996). The importance of shading in regulating benthic algal biomass was first recognised in the River Continuum Concept (Vannote *et al.*, 1980) which proposed a downstream pattern of increasing biomass as channels widened and riparian shading was reduced.

The thermal regime of rivers has been cited as being important in maintaining overall stream health (Caissie, 2006). Shading has been shown to reduce stream temperatures, especially maximum temperatures (Bowler *et al.*, 2012). Planting trees along riparian corridors can have further positive effects both to the in-stream ecology and the wider ecosystem. For example, trees can stabilise river banks, thus reducing erosion, provide habitat and refugia for a number of species as well as alter the in-stream nutrient environment (Lenane, 2012). Tree roots can act as a nutrient sink, removing nutrients from the environment while leaves of trees can act as a nutrient source when they decompose. When considering shading levels to rivers, it is important to achieve a balance between having light intensities that will limit periphyton growth and having enough light for in-stream macrophytes such as *Ranunculus* sp. to thrive (Lenane, 2012).

3.1.1 The light – nutrient hypothesis

The importance of light intensity and its interaction with nutrients in affecting periphyton growth within streams has been recognised in the form of the light –

nutrient hypothesis. First proposed by Urabe and Sterner (1996) and elaborated upon by Sterner *et al.* (1997), this states that as light intensity increases, the nutritional content of algae (periphyton) and, therefore, their quality (biochemical composition) as a food resource to grazers and other invertebrates decreases (Figure 3.1). Although Urabe and Sterner (1996) and Sterner *et al.* (1997) proposed the light – nutrient hypothesis in terms of phosphorus: carbon ratio, subsequent studies have always interpreted results in terms of carbon: phosphorus ratio (Urabe *et al.*, 2002, Hall *et al.*, 2004, Dickman *et al.*, 2006, Hall *et al.*, 2007, Dickman *et al.*, 2008, Faithfull *et al.*, 2011).

Assuming river phosphorus concentration is constant; periphyton biomass will initially increase as light intensity increases, since at low light intensities, periphyton growth is limited by irradiance. Eventually, light intensity is saturating for growth and periphyton growth becomes limited by a secondary factor (for example, phosphorus concentration) so that biomass reaches a plateau and there is no further increase (Figure 3.1). Simultaneously, the carbon: phosphorus ratio will increase as light intensity increases. At low light levels, phosphorus supply is sufficient relative to biomass. However, as light intensity increases, so does periphyton biomass and the proportion of carbon (energy), leading to an increase in the carbon: phosphorus ratio (Figure 3.1). The increase in ratio is reflected as a decrease in quality and quantity of the periphyton as a food resource (Urabe and Sterner, 1996).

In general, periphyton stoichiometry (carbon: nutrient ratios) reflects the supply of light and nutrients available to the periphyton cells (Sterner *et al.*, 1997, Hall *et al.*, 2007). In natural stream ecosystems, the balance between photosynthesis and nutrient uptake may therefore exert a bottom up control on productivity (Dickman *et al.*, 2006, Dickman *et al.*, 2008) and can control the community composition of zooplankton species (Urabe *et al.*, 2002, Hall *et al.*, 2004).

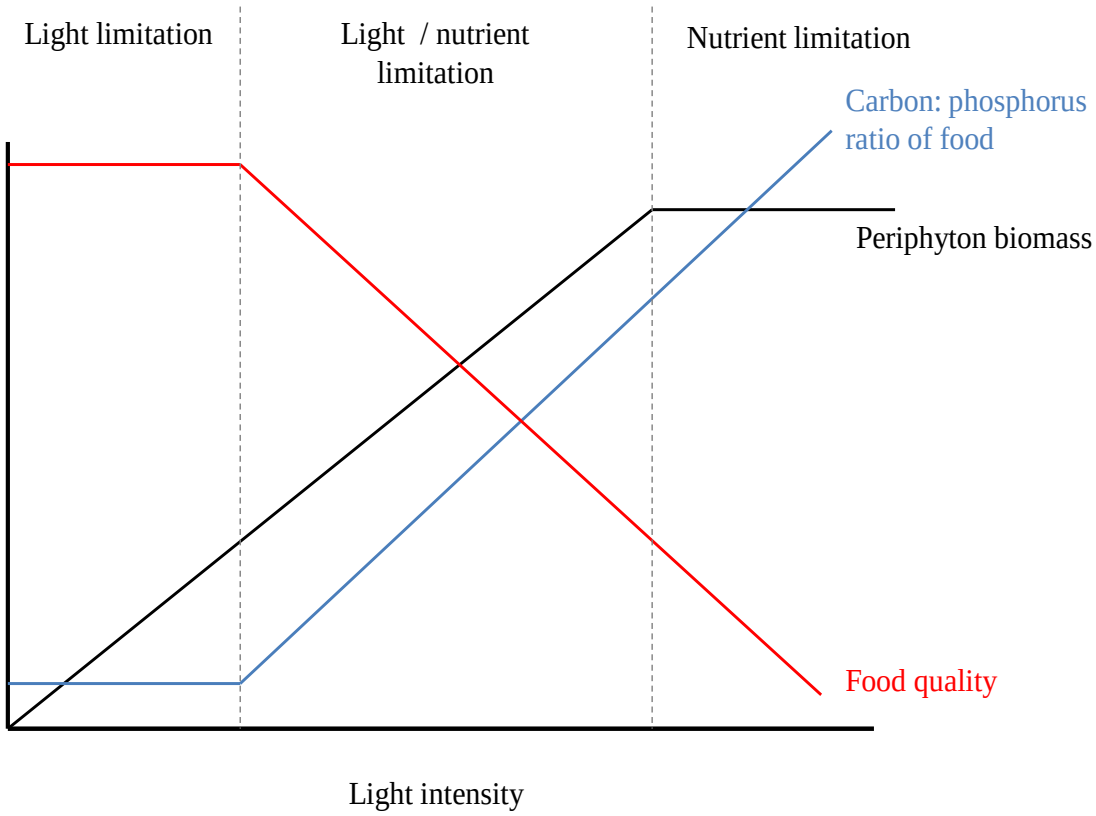


Figure 3.1: Schematic diagram showing how periphyton biomass, nutrient ratio and food quality change when light intensity increases while nutrient (phosphorus) concentrations are fixed. Adapted from Urabe and Sterner (1996).

3.1.2 Experimental aims and hypotheses

Previous flume experiments have investigated the impact of changing phosphorus concentrations on periphyton growth rates and accrual (Bowes *et al.*, 2007, Bowes *et al.*, 2010a, Bowes *et al.*, 2012a) across a range of rivers (the River Frome, River Kennet and River Thames) of varying levels of nutrient enrichment in the south of England. Average ambient SRP concentrations in these experiments ranged from 60 to 225 µg l^{-1}. The River Kennet (SRP = 60 µg l^{-1}) (Bowes *et al.*, 2010a) and River Frome (SRP = 90 µg l^{-1}) (Bowes *et al.*, 2007) were at the phosphorus-limiting threshold (or saturation point), meaning that increases in SRP concentration had no effect on periphyton accrual rate, but decreases in SRP concentration resulted in decreased periphyton accrual. The ambient SRP concentration of the River Thames was above the phosphorus-limiting threshold. Reducing SRP concentration in the River Thames (SRP = 225 µg l^{-1}) by iron dosing suggested that SRP concentrations needed to be reduced to below100 µg l^{-1} before periphyton accrual rate was reduced

(Bowes *et al.*, 2012a). The study on the River Thames also showed shading to have a significant effect in reducing periphyton growth rate. The same study suggested that it was not until SRP concentrations were reduced to below 30 µg l^{-1} that a change in diatom community assemblage was observed (Bowes *et al.*, 2012a).

The River Lambourn is a chalk stream in West Berkshire (further details are given in Section 3.1.3). The Centre for Ecology and Hydrology (CEH) has collected weekly water chemistry samples from this river (at CEH's Lambourn Observatory site at Boxford) since June 2008 (Figure 3.2, sampling site is Site 5 in the longitudinal survey see Figure 3.3). The data shows that the river has relatively good water quality for south-east England. Ambient SRP concentrations ranged from 15 to 83 µg l^{-1} with a mean value of 40 µg l^{-1} (Figure 3.2).

Therefore, this experiment aimed to determine (based on the conclusions of Bowes *et al.*, 2007, Bowes *et al.*, 2010 and Bowes *et al.*, 2012a) whether the River Lambourn, with its lower SRP concentrations, was truly phosphorus limited. The effect of shading was simultaneously investigated in order to verify the light – nutrient hypothesis and determine the interaction and relative importance of light and nutrients in limiting periphyton growth.

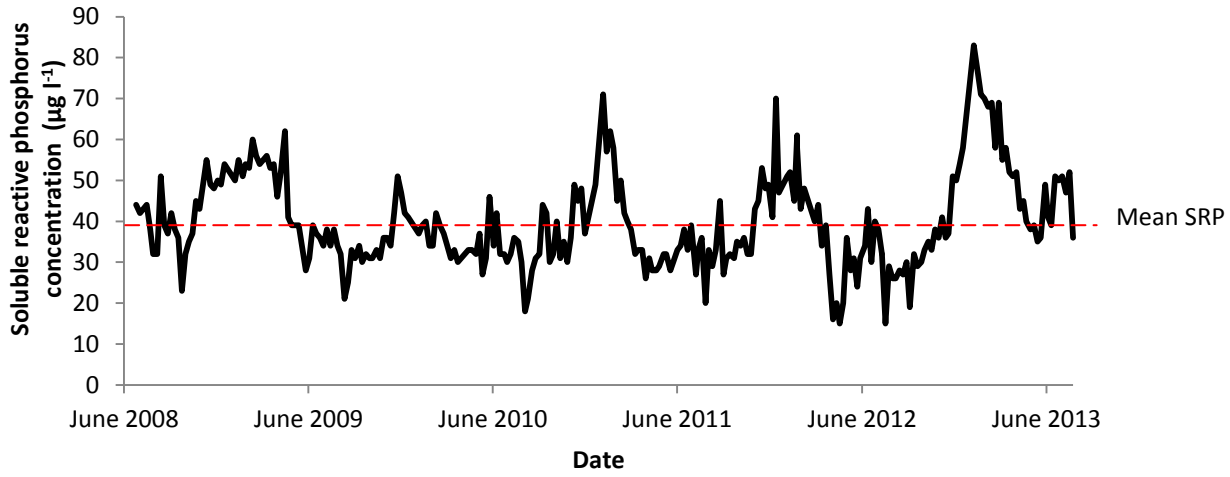


Figure 3.2: Soluble reactive phosphorus (SRP) concentrations from weekly sampling at Boxford (Site 5 of longitudinal survey) between June 2008 and June 2013. The red dashed line shows the mean SRP concentration of the five year dataset. (Data source – CEH Lambourn Observatory monitoring programme).

The specific hypotheses to be tested (at a significance level of 0.05) were:

1. **H_1** – Manipulating nutrient concentrations will result in significant differences in periphyton accrual as indicated by changes in chlorophyll-*a* concentration, AFDM, AI and carbon: nutrient ratios, as well as a shift in community composition of the periphyton biofilm in flumes as indicated by TDI and flow cytometry regardless of light intensity.

   H_0 – There is no significant difference between nutrient concentrations and periphyton accrual.
2. **H_1** – At each nutrient concentration, there will be significant differences between chlorophyll-*a* concentration, AFDM, AI and carbon: nutrient ratios in unshaded and shaded sections of each flume. Shading will lead to a decrease in periphyton accrual.

   H_0 – There is no significant difference in chlorophyll-*a* concentration, AFDM, AI and carbon: nutrient ratios between unshaded and shaded sections of each flume.

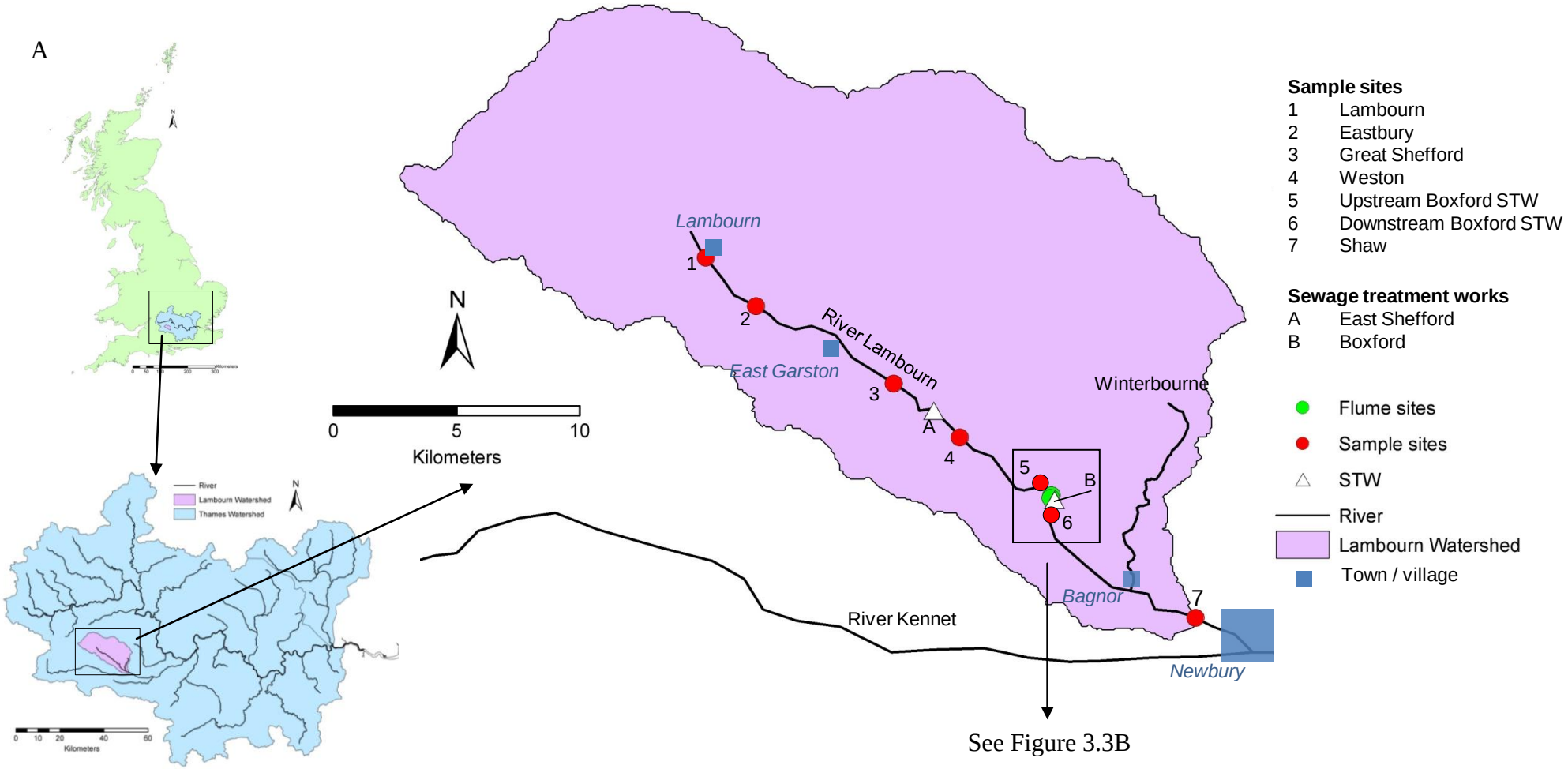
3.1.3 Catchment description and study site

The River Lambourn is a chalk stream located in West Berkshire, England (Figure 3.3A). The source of the river is located 152 m above sea level in Lynch Wood, to the north of the village of Lambourn (English Nature, 1995). The 25 km river flows in a south easterly direction through the villages of Lambourn, Eastbury, East Garston, Great Shefford, Weston and Boxford with a total catchment area of 234.1 km^2 (Marsh and Hannaford, 2008). The perennial head of the river is located at Great Shefford, approximately 8 km downstream from the source.

The River Lambourn is a tributary of the River Kennet which itself is a tributary of the River Thames. The confluence of the Lambourn and the Kennet is to the east of Newbury while the Kennet joins the Thames at Reading (Figure 3.3A). The only significant tributary of the River Lambourn is the Winterbourne which joins the river near to Bagnor (Neal et al., 2004a). The high base flow index of the River Lambourn (0.97) is typical of rivers dominated by groundwater (Marsh and Hannaford, 2008). The mean flow of the river at Shaw, near to its confluence with the Kennet (Site 7 on

Figure 3.3A), between October 1962 and December 2011 was 1.728 m^3 s^{-1} (Marsh and Hannaford, 2008).

The entire chalk river was designated as a site of special scientific interest in 1995 due to it being a classic example of a lowland chalk river (English Nature, 1995) with a number of species being found within it having specialist protection under the Biodiversity Action Plan. The river is home to a number of nationally scarce invertebrate species including the predatory flatworm, *Crenobia alpina;* the beetle, *Rhantus suturalis* and the caddis flies, *Matelype fragilis* and *Ylodes conspersus*. In addition, the river supports large populations of wild brown trout, *Salmo trutta* and the grayling species, *Thymallus thymallus* (English Nature, 1995).


A
N
0
5
10
Kilometers
Lambourn
East Garston
River Lambourn
Winterbourne
Bagnor
River Kennet
Newbury
1
2
3
A
4
5
B
6
7
River
Lambourn Watershed
Thames Watershed
0 10 20 40 60
Kilometers
Sample sites
1 Lambourn
2 Eastbury
3 Great Shefford
4 Weston
5 Upstream Boxford STW
6 Downstream Boxford STW
7 Shaw
Sewage treatment works
A East Shefford
B Boxford
Flume sites
Sample sites
STW
River
Lambourn Watershed
Town / village
See Figure 3.3B

B

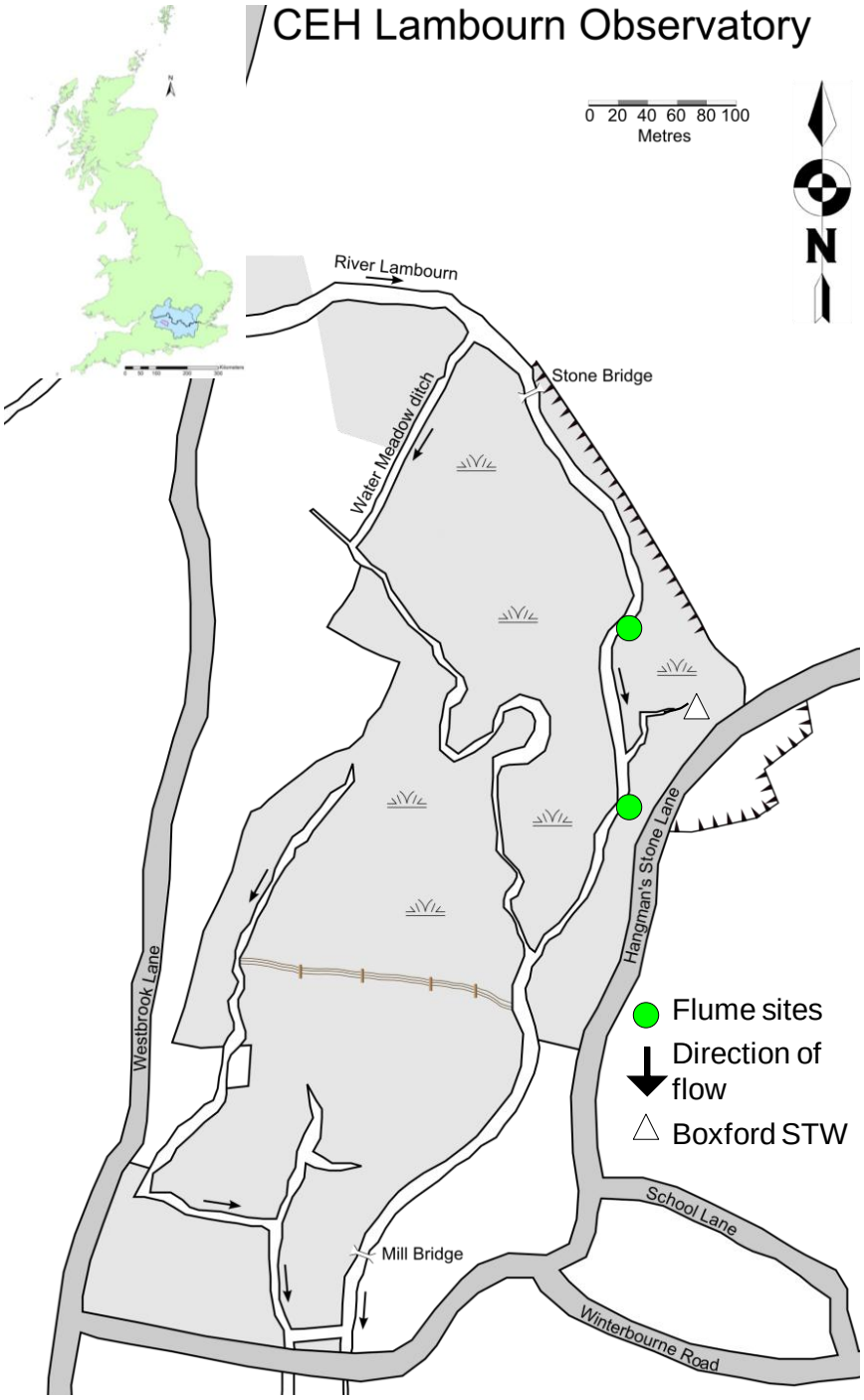


Figure 3.3: Map of the River Lambourn study sites showing (A) the longitudinal survey sites, sewage treatment works and towns / villages on the River Lambourn, placing them into context of the wider catchment area and the UK and (B) the flume experiment location.

3.2 Experiment-specific methodology (Experiment 1)

Further details on the methods used are found in Sections 2.1 to 2.3. Fifteen in-stream flume mesocosms (five sets of three) were deployed (Figure 3.4) on the River Lambourn at the southern most flume site (Figure 3.3B) during mid-April 2012. The flow velocity gates were set so the water velocity within each flume at the start of the experiment was 0.15 m s^{-1}. Nutrient / iron dosing treatments and target nutrient concentrations for each flume are given in Table 3.1. A concentration-effect approach was chosen over treatment replication in order to accurately identify the concentration at which phosphorus became limiting in the River Lambourn (Guckert, 1993, Bowes *et al.*, 2012a). The distance between the different sets of flumes was a minimum of 3 m (Figure 3.4), which was sufficient to prevent nutrient contamination between them. Flumes were secured in place using scaffolding poles that were pile driven into the river bed. Experiment 1 ran from 24th April to 5th May 2012.

Figure 3.4: Photograph of how the 15 flumes were positioned for Experiment 1 in the River Lambourn. The blue arrow represents direction of river flow.

Table 3.1: Target nutrient concentrations during Experiment 1 on the River Lambourn from 24th April to 5th May 2012. Increases and decreases were based on an ambient SRP concentration of 40 µg l^{-1} and NO_3-N concentration of 7 mg l^{-1}.

Flume number	Nutrient treatment	Target increase or decrease in SRP concentration (%)	Target increase in NO_3-N concentration (%)
1	$FeSO_4$ addition	- 40	N/A
2	None (control)	N/A	N/A
3	P addition	75	N/A
4	$FeSO_4$ addition	- 10	N/A
5	P addition	75	N/A
6	None (control)	N/A	N/A
7	$FeSO_4$ addition	- 25	N/A
8	None (control)	N/A	N/A
9	P addition	175	N/A
10	None (control)	N/A	N/A
11	PN addition	75	20
12	N addition	N/A	20
13	P addition	200	N/A
14	PN addition	200	20
15	None (control)	N/A	N/A

3.2.1 Light intensity

In order to mimic tree shading (Bowes *et al.*, 2012a), three layers of greenhouse shade cloth were attached to a wire mesh frame that was positioned over the lower sections of the flumes, so that half of the periphyton monitoring area was shaded and half was in full sun (Figure 3.5). The shade cloth was positioned so that tiles remained in its shadow regardless of time of day. HOBO pendant loggers were placed in a shaded and unshaded part of the flume to record temperature and light intensity at hourly intervals.

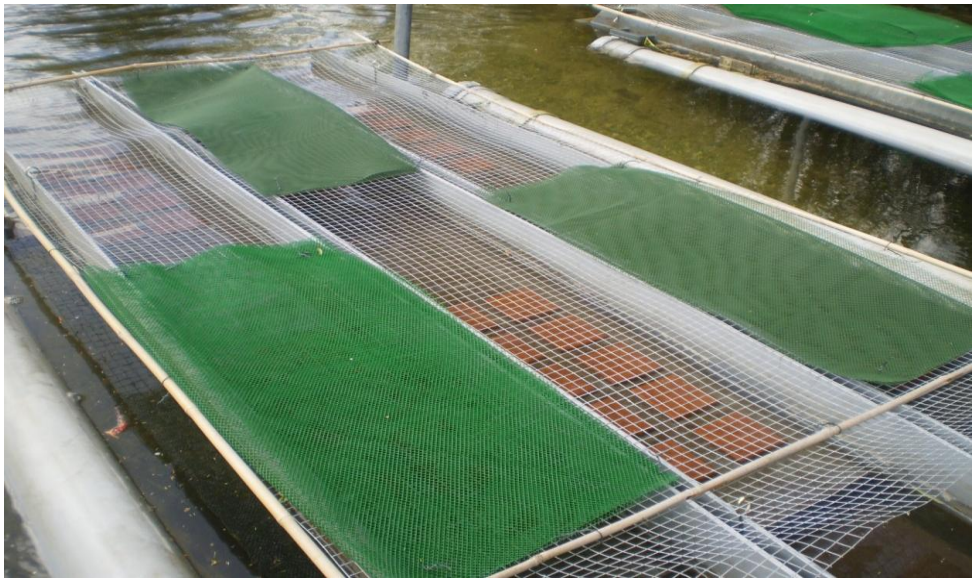


Figure 3.5: Shaded and unshaded sections of flume in the River Lambourn.

3.3 Experiment 1 results and discussion

3.3.1 Light intensity and temperature

Shading sections of each flume with greenhouse shade cloth significantly reduced the light intensity in that section of the flume. The average maximum daily light intensities during daylight hours were 51 228 Lx and 18 631 Lx for unshaded and shaded treatments, respectively, a reduction of 64 %. Immediately before the beginning of the experiment, on a clear day, light intensity was measured in full light, under dappled tree cover and under full tree cover across the study site. Dappled tree cover and full tree cover were found to reduce light intensities by *ca.* 64 % and 82 % respectively. Therefore, in Experiment 1, the flume shading provided realistic reductions in light intensity, equivalent to dappled tree cover. Shading had little effect on water temperature and mean temperatures in the flumes were 10.64 ± 3.49 °C and 10.44 ± 3.25°C for the unshaded and shaded treatments, respectively. For the duration of the experiment, the mean River Lambourn temperature was 10.52 ± 5.24 °C, suggesting that shading did not affect river water temperature.

3.3.2 Nutrient treatments and periphyton response

The average SRP concentrations measured in the flumes are shown in Figure 3.6. Four flumes received phosphorus addition which increased mean SRP concentration to between 78.8 and 126.3 µg l^{-1}. Two flumes received a combined phosphorus and nitrogen addition which increased mean SRP concentration to 97.6 and 132.5 µg l^{-1}. The nitrogen concentration in these flumes was increased to *ca.* 7.5 mg l^{-1}. One flume received nitrogen addition, increasing concentration to 7.43 mg l^{-1}. A further three flumes were dosed with iron, reducing mean SRP concentrations to between 29.7 and 46.4 µg l^{-1}. The remaining five flumes had unmodified river water flowing through them. The mean SRP concentrations of these over the course of the 11 day experiment were between 41.3 and 52.9 µg l^{-1} and nitrogen concentrations were between 6.40 and 7.16 mg l^{-1}.

There was a lot of overlap between phosphorus treatments, especially between the control and phosphorus reduction (iron addition) treatments. The control flumes had a wide range of SRP concentrations (20.9 to 87.2 µg l^{-1}) with intermittent phosphorus spikes (e.g. 30th April and 1st May 2012). On each individual sampling occasion, it was expected that the SRP concentration in the five control flumes would be similar. However, this was not always the case. For example, at 10:30 am on 24th April 2012, the SRP concentrations for Flume 2, 6, 8, 10 and 15 (all control flumes) were 53.9, 52.0, 29.4, 61.6 and 41.6 µg l^{-1} (a range of 32.2 µg l^{-1}). Yet, at 10:00 am on 4th May 2012, the range in SRP concentration between these five flumes was only 4.2 µg l^{-1}. As a result, the means of the control treatments showed variation (41.3, 47.8, 47.8, 48.3 and 52.9 µg l^{-1}) and were greater than what was expected based on the long-term monitoring at the CEH Lambourn Observatory (Figure 3.2).

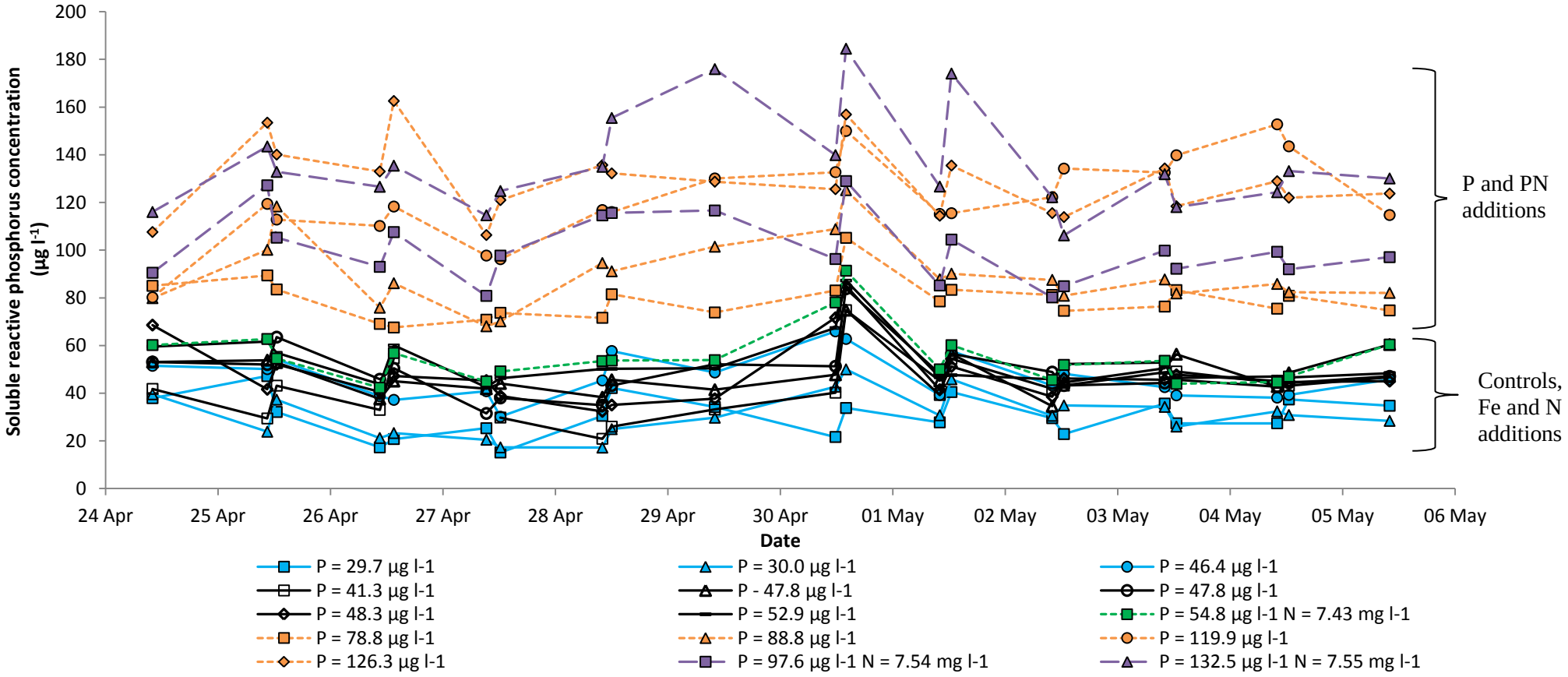


Figure 3.6: Soluble reactive phosphorus (SRP) concentrations observed in each flume over the course of the 11 day nutrient manipulation experiment. Solid blue line with filled symbol = iron addition (phosphorus reduction), solid black line with open symbol = control (no addition), dotted green line with filled symbol = nitrogen addition (no phosphorus), dotted orange line with filled symbol = phosphorus addition (no nitrogen) and dashed purple line with filled symbol = phosphorus and nitrogen addition.

Shading had a significant effect in reducing periphyton biomass across all nutrient treatments (Figure 3.7 and Figure 3.8), with growth rate reductions of *ca*. 50 %. However, the overlap in nutrient treatments and spikes in phosphorus concentration affected periphyton biomass response to nutrients as evidenced in Figure 3.7A and Figure 3.8A. The experiment was designed to test whether at the low SRP concentrations of the River Lambourn, phosphorus was truly limiting. However, it was not possible to test this in the current experiment (due to the reasons outlined below).

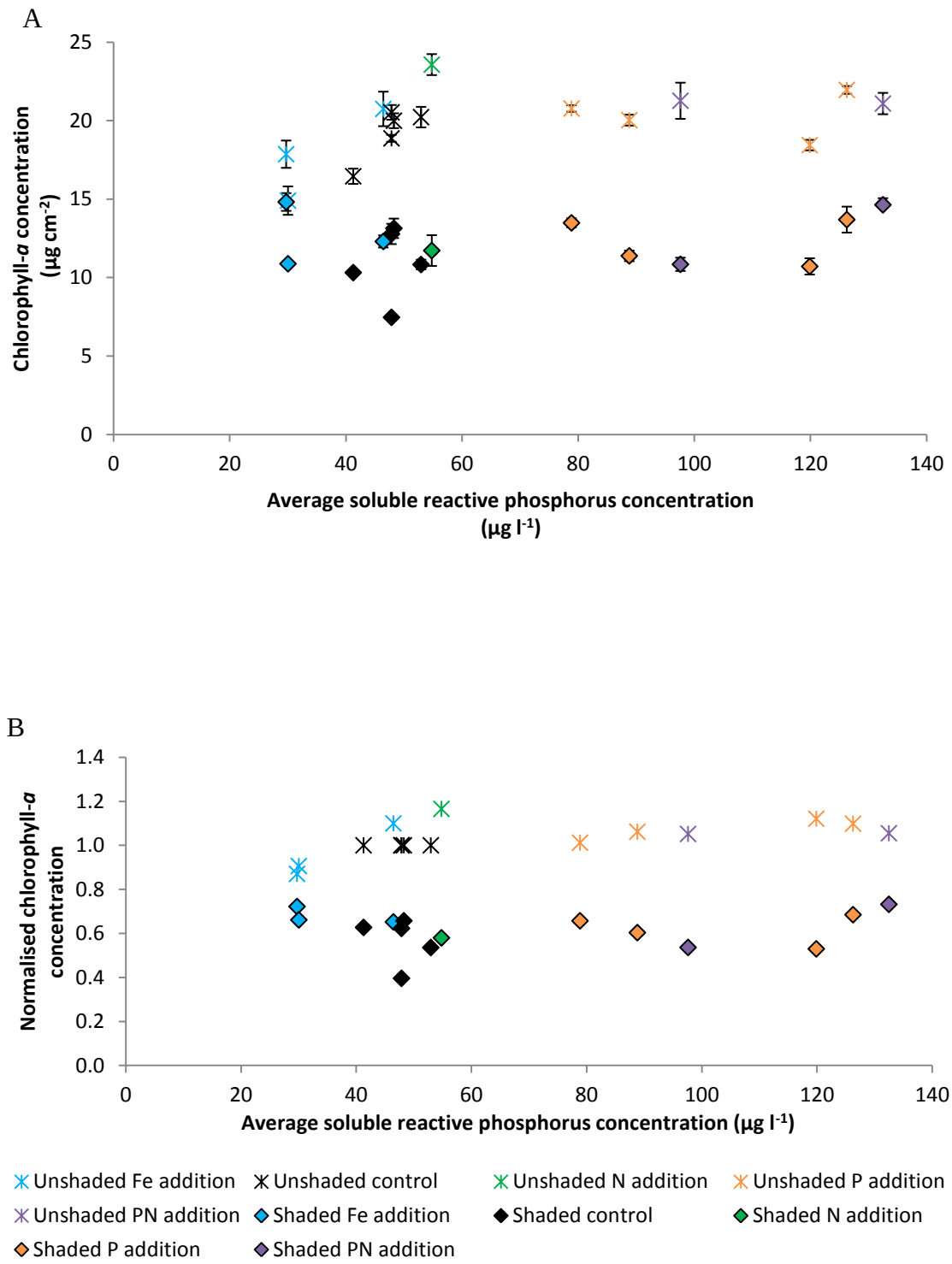


Figure 3.7: Relationship between soluble reactive phosphorus (SRP) concentration and (A) chlorophyll-*a* concentration (data points are mean values based on analysis of three tiles ± one standard error) and (B) normalised chlorophyll-*a* concentration on the final day of the flume experiment at both light levels.

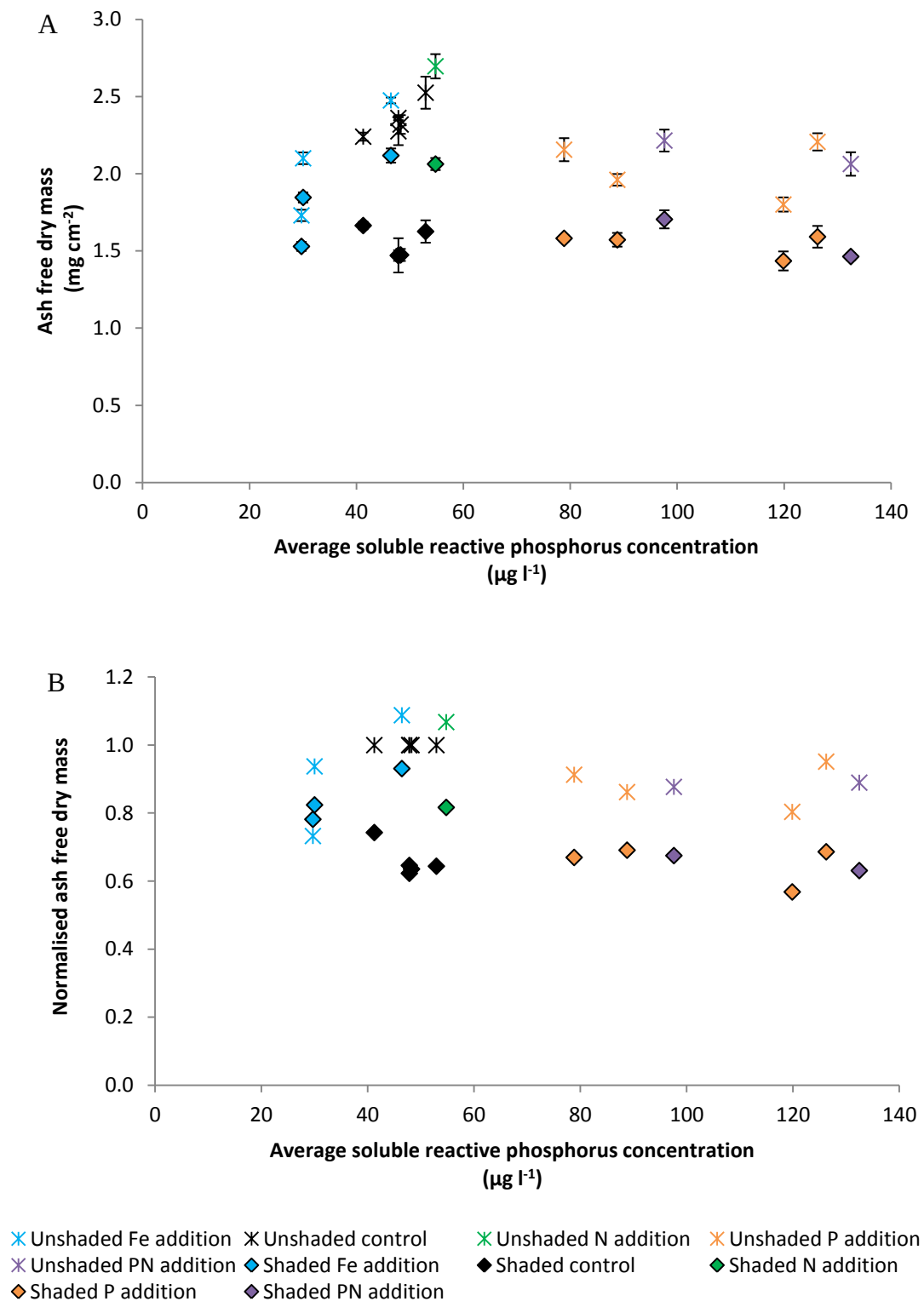


Figure 3.8: Relationship between soluble reactive phosphorus (SRP) concentration and (A) ash free dry mass value (data points are mean values based on analysis of three tiles ± one standard error) and (B) normalised ash free dry mass value on the final day of the flume experiment at both light levels.

3.3.3 Effects of Boxford sewage treatment works

The experimental site was located 50 m downstream of Boxford STW (Figure 3.3A and B). As this was relatively small (population estimate of 350), it was thought that it would not affect the experiment. However, SRP concentrations measured in the control flumes (ambient river water) at the time of the experiment were higher than expected based on the CEH River Lambourn long-term dataset, which is collected *ca.* 300 m upstream of the STW. Also, the control flume SRP concentrations showed great variation during the flume water sampling (Figure 3.6). This would suggest that the STW was increasing nutrient levels in the River Lambourn, and also intermittently producing large spikes in SRP concentration, which were adversely affecting the experiment.

To test this, simultaneous samples were collected from the river, 10 m upstream and 20 m downstream of the STW outfall. Water samples were collected at five minute intervals for one hour on 2nd May 2012. These were immediately filtered through 0.45 µm glass fibre filter papers and analysed for SRP in the field (for method see Section 2.4.2). The results of this analysis are presented in Figure 3.9.

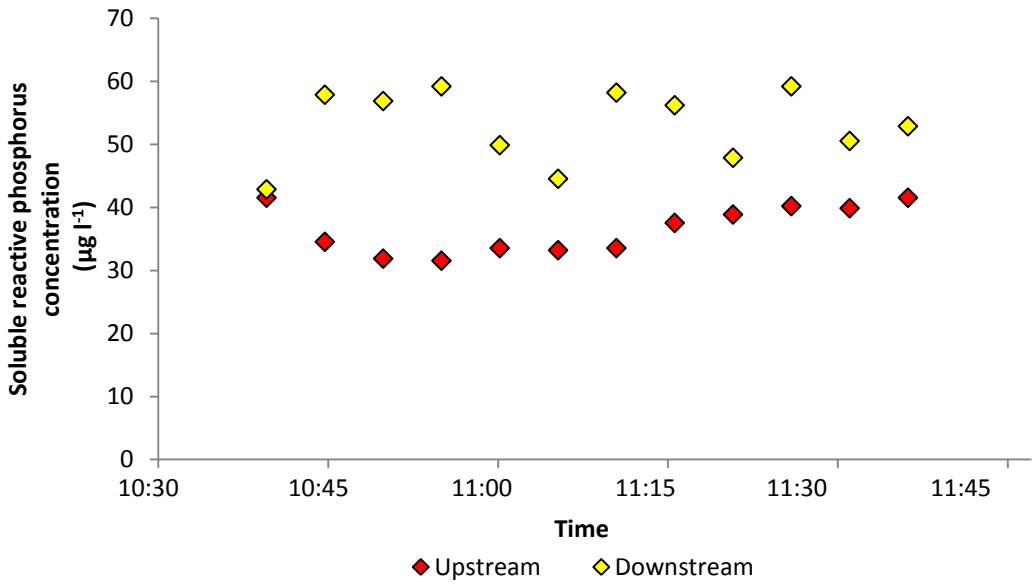


Figure 3.9: Soluble reactive phosphorus concentrations over the course of one hour, upstream and downstream of the sewage treatment works effluent input to the River Lambourn at Boxford.

The mean SRP concentrations were 36.5 and 53.0 µg l^{-1} upstream and downstream of the STW respectively. The SRP concentration downstream was much more variable than that upstream. After testing for normality, a two sample T-test was run which found there to be significant differences in SRP concentration in the River Lambourn upstream and downstream of the STW input (T value: -8.25, $p < 0.001$) suggesting the effluent input from the STW was increasing SRP concentrations in the section of river immediately downstream of it. This explains the limited control and wide variation in phosphorus concentrations in Experiment 1.

3.3.4 Raindrop impact

As well as the unexpected influence from the STW, Experiment 1 was affected by a severe overnight storm on 1st May 2012 which caused disturbance to the periphyton biofilms on some tiles due to raindrop impact (Figure 3.10). The effects of this are evident in Figure 3.7B and Figure 3.8B. Normalising the values for treatment flumes to the unshaded control in each set of three flumes should result in the control values all being the same. However, in Experiment 1, this was not the case which made it difficult to draw accurate quantitative conclusions from the data collected.

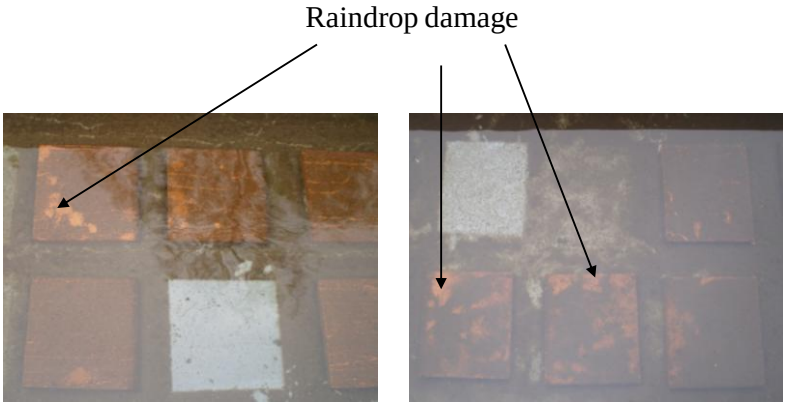


Figure 3.10: Storm damage to periphyton biofilms on artificial substrates.

3.3.5 Elemental stoichiometry

The raindrop damage meant it was not possible to quantitatively examine the effects of nutrient limitation on periphyton biomass in Experiment 1. However, as elemental stoichiometry is a qualitative technique, it was possible to examine the effect of light on the quality of periphyton as a food resource for potential grazing invertebrates (the light-nutrient hypothesis – see Section 3.1.1 and Urabe and Sterner (1996)). The majority of studies conducted to examine the light - nutrient hypothesis have been undertaken on lake ecosystems (Sterner *et al.*, 1997, Hall *et al.*, 2004, Dickman *et al.*, 2006, Hall *et al.*, 2007, Dickman *et al.*, 2008, Faithfull *et al.*, 2011) with only limited research being undertaken in river ecosystems (Fanta *et al.*, 2010, Hill *et al.*, 2010).

At each nutrient treatment, the C: P ratio was lower in the shaded flumes compared to the unshaded flumes (Figure 3.11), representing a periphyton community that is of higher quality as a food resource to grazing invertebrates and is consistent with results reported previously (Urabe *et al.*, 2002, Hall *et al.*, 2004, Dickman *et al.*, 2006, Dickman *et al.*, 2008). The quality of periphyton as a food source is important to overall ecosystem function as grazing invertebrates obtain elements necessary for life processes from their food. Poor quality food has been shown to reduce the biomass of invertebrate species (specifically *daphnia* sp. which have a high phosphorus requirement) (Urabe *et al.*, 2002, Hall *et al.*, 2004). Shaded systems have also been shown to be more efficient with lower light increasing 'food chain efficiency.' Food chain efficiency is defined as the proportion of energy fixed by primary producers that is transferred to the top trophic level (Dickman *et al.*, 2008).

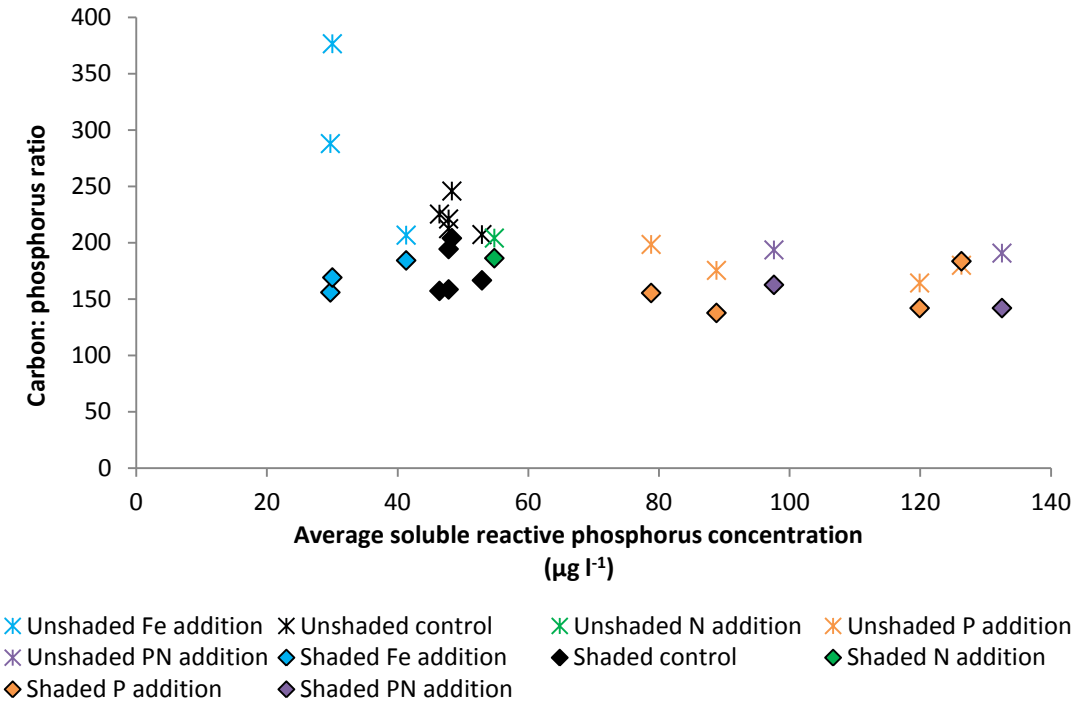


Figure 3.11: Relationship between soluble reactive phosphorus concentration and carbon: phosphorus ratio.

Contrary to the work of others (Dickman *et al.*, 2006, Dickman *et al.*, 2008, Faithfull *et al.*, 2011), nutrient enrichment in the flumes had no effect on carbon: phosphorus ratio and food quality in the shaded treatments (Figure 3.11) suggesting nutrient concentrations were at or above the phosphorus-limiting threshold. However, in the unshaded treatment, there was a nutrient response in carbon: phosphorus ratio suggesting an interaction between nutrients and light which could affect the threshold (i.e. the phosphorus-limiting threshold is light dependent). Decreasing phosphorus concentration resulted in much higher carbon: phosphorus ratios of up to 377: 1 in unshaded treatments (Figure 3.11) indicating a decrease in quality of periphyton as a food resource (Figure 3.1). This is in agreement with the light–nutrient hypothesis which states food quality is lowest at high light and low nutrient concentrations (Urabe and Sterner, 1996, Sterner *et al.*, 1997). The fact that the largest difference in carbon: phosphorus ratio between shaded and unshaded treatments was at the lowest phosphorus concentration agrees with the findings of Urabe *et al.* (2002) and shows the importance of shading at low nutrient concentrations to maintain food quality.

The elemental analysis of periphyton biofilms can have important policy implications in terms of the UK meeting the requirements of the Water Framework Directive (WFD). The majority of UK policy is focussed on the effects of reducing phosphorus concentration to improve ecological status (UKTAG, 2013a). However, as Figure 3.7 and Figure 3.8 show, shading significantly reduces periphyton biomass, regardless of nutrient treatment. Furthermore, shading helps maintain food quality of periphyton at reduced SRP concentrations. Without shading (i.e. in full light) food quality is reduced at lower SRP concentrations as indicated by a higher carbon: phosphorus ratio (Figure 3.11). Therefore, the simple addition of riparian vegetation to shade streams results in a lower periphyton accrual rate that is of higher nutritional quality. Shading will not only benefit grazing invertebrates but will also benefit macrophyte species that compete with periphyton for light.

3.4 Experimental design of Experiment 2

Experiment 1 showed riparian shading to have a positive effect in reducing periphyton biomass and altering elemental stoichiometry, but due to the combined effects of Boxford STW and storm damage (Sections 3.3.3 and 3.3.4) nutrient effects were masked. To quantitatively test whether phosphorus was truly limiting at the ambient SRP concentration in the River Lambourn and to have a better control of phosphorus concentration in the flumes, nine flumes were moved to an unshaded section of river upstream of the STW input (so not to be affected by it) for Experiment 2 (Figure 3.3B). Six flumes were left at the initial downstream site to examine whether a slightly higher ambient SRP concentration (*ca.* 50 µg l^{-1}, compared with *ca.* 35 µg l^{-1} upstream) affected periphyton colonisation and biomass accrual. This allowed phosphorus concentration to be reduced to *ca.* 35 µg l^{-1}, to determine if the phosphorus-limiting threshold for the River Lambourn was lower than the ambient concentration experienced at the downstream site. It also allowed the effects of temporarily adding phosphorus to periphyton biofilm communities then removing it to be observed.

As Experiment 1 did not show large differences in measures of periphyton biomass with increasing nutrient concentrations (Figure 3.7 and Figure 3.8) Experiment 2 largely focussed on the effects of reducing SRP concentrations below the ambient

concentration. The first experiment successfully used the iron sulphate phosphorus-stripping methodology of Bowes *et al.* (2007). After examining the effects of different forms of iron in reducing SRP concentrations (Appendix A), it was decided that in Experiment 2 and for future experiments, iron (III) chloride ($FeCl_3$) would be used. Iron (III) chloride was found to be more effective at reducing SRP concentration, dissolved more easily in the deionised water and has been successfully used in STW to reduce phosphorus concentrations for a number of decades (Yeoman *et al.*, 1988). The target nutrient concentrations at each site are given in Table 3.2.

Table 3.2: Target nutrient concentrations during Experiment 2 on the River Lambourn from 10th to 21st May 2012. The abbreviations US and DS after flume number stand for upstream and downstream of Boxford sewage treatment works respectively. Increases and decreases were based on an ambient SRP concentration of 40 µg l^{-1} and NO_3 – N concentration of 7 mg l^{-1}.

Flume number	Nutrient treatment	Target increase or decrease in SRP concentration (%)	Target increase in NO_3-N concentration (%)
1US	P addition	250	N/A
2US	PN addition	200	10
3US	None (control)	N/A	N/A
4US	$FeCl_3$ addition	- 40	N/A
5US	$FeCl_3$ addition	- 30	N/A
6US	None (control)	N/A	N/A
7US	P addition	250	N/A
8US	None (control)	N/A	N/A
9US	$FeCl_3$ addition	- 50	N/A
1DS	P addition	200	N/A
2DS	None (control)	N/A	N/A
3DS	$FeCl_3$ addition	- 40	N/A
4DS	None (control)	N/A	N/A
5DS	$FeCl_3$ addition	- 30	N/A
6DS	$FeCl_3$ addition	- 50	N/A

Experiment 1 showed three layers of greenhouse shade cloth mimicked dappled tree shading and this successfully reduced periphyton accrual (Figure 3.7 and Figure 3.8). As a result, the density of shade cloth was unchanged in Experiment 2. The position of shading within each flume (in the upstream or downstream section) at both the

upstream and downstream site was randomly assigned (Figure 3.12). As in Experiment 1, light and temperature within unshaded and shaded sections of the flume and temperature of the River Lambourn were recorded hourly throughout the experiment. The flow velocity gates were set so the water velocity within each flume at the start of Experiment 2 was 0.15 m s^{-1}. Experiment 2 was also 11 days long and ran from 10th to 21st May 2012.

Figure 3.12: Photographs of how the flumes were positioned at (A) the downstream and (B) the upstream site during Experiment 2 on the River Lambourn. The blue arrow represents direction of river flow.

3.4.1 Periphyton analysis methods

In addition to measures of chlorophyll-*a* concentration, AFDM and diatom identification / TDI calculation (Section 2.2), flow cytometry analysis was performed on periphyton biofilm samples grown in each flume. Flow cytometry is a laser based analytical technique that discriminates particles within the periphyton biofilm based on size, shape, cell structure and their constituent photosynthetic pigments. It therefore makes it possible to determine the relative proportions of nanoeukaryotes (green algae), diatoms, cryptophytes (red algae) and cyanobacteria (blue green algae) within the biofilm. A full description of the flow cytometry method is given elsewhere (Read *et al.*, 2014).

On the final day of the experiment, one tile from the shaded and unshaded section of each flume was removed. This tile was scrubbed thoroughly with a toothbrush to remove all traces of the biofilm. The resulting suspension was rinsed into a 50 ml centrifuge tube using deionised water. Upon return to the laboratory, the suspension was homogenised by shaking and 500 µl passed through a 40 µm cell strainer (BD Biosciences, Oxford, UK) to remove large sediment. After which, 20 µl of the filtrate was added to 980 µl of ultrapure water in a flow cytometry tube. The sample was loaded into the carousel and analysed using a Beckman-Coulter Gallios flow cytometer (Beckman-Coulter, High Wycombe, UK) equipped with blue (488 nm) and red (638 nm) solid state diode lasers. Two scatter plots were used to delineate and count the major periphyton types (diatoms, green algae and cyanobacteria).

The first plot was of yellow/green fluorescence (575 nm) on the x-axis against red fluorescence (695 nm) on the y-axis, both excited by the 488 nm laser. This represents a plot of phycoerythrin versus chlorophyll. The second plot was used to identify and count groups of phycocyanin-containing cyanobacteria. To achieve this, a plot of red fluorescence (695 nm) excited by the 488 nm laser on the x-axis versus orange fluorescence (660 nm) excited by the 635 nm laser on the y-axis (chlorophyll versus phycocyanin) was used. Samples were run for one minute at the high speed setting (approx 50 µl per minute). To prevent decreases in cell numbers due to cells dying, samples were not preserved and were run within 24 hours of collection.

3.5 Experiment 2 results and discussion

3.5.1 Light intensity and temperature

For the duration of the experiment, tile substrates in both light treatments (unshaded and shaded) received light for 15 hours per day, between the hours of 6:00 am to 9:00 pm. Shading significantly reduced the amount of light reaching the tiles (Figure 3.13A) while location (upstream or downstream of the STW input) did not affect light intensity. The tiles in the unshaded flumes received maximum daily light intensities of between 26 178 and 214 535 Lx throughout the course of the 11 day experiment. The average maximum daily light intensity was 100 837 Lx. The average light intensity during daylight hours was 36 415 Lx. The flumes that were shaded received maximum light intensities between 8 267 and 45 867 Lx. The average maximum daily light intensity in the shaded flumes was 22 152 Lx, while the average light intensity during daylight hours was 6 641 Lx. Shading reduced mean maximum daily light intensity by 78 %, equivalent to full tree shading.

The hourly temperature measurements recorded in unshaded and shaded sections of flume upstream and downstream of the STW input and the River Lambourn throughout the 11 day experiment are shown in Figure 3.13B. A two sample T-test between flumes at the same light level showed that location did not affect temperature (unshaded: $T = -0.57$, $p = 0.567$; shaded: $T = 0.807$, $p = 0.940$). Mean temperatures in the unshaded and shaded flumes were 11.25 ± 2.98 °C and 11.14 ± 2.91 °C respectively. For the duration of the experiment, the mean temperature of the River Lambourn (downstream of STW input) was 11.30 ± 2.72 °C, with a minimum temperature of 8.58 °C and a maximum temperature of 13.75 °C (Figure 3.13B). The temperature difference between unshaded and shaded sections of flume was insignificant (two sample T-test: $T = -1.690$, $p = 0.090$).

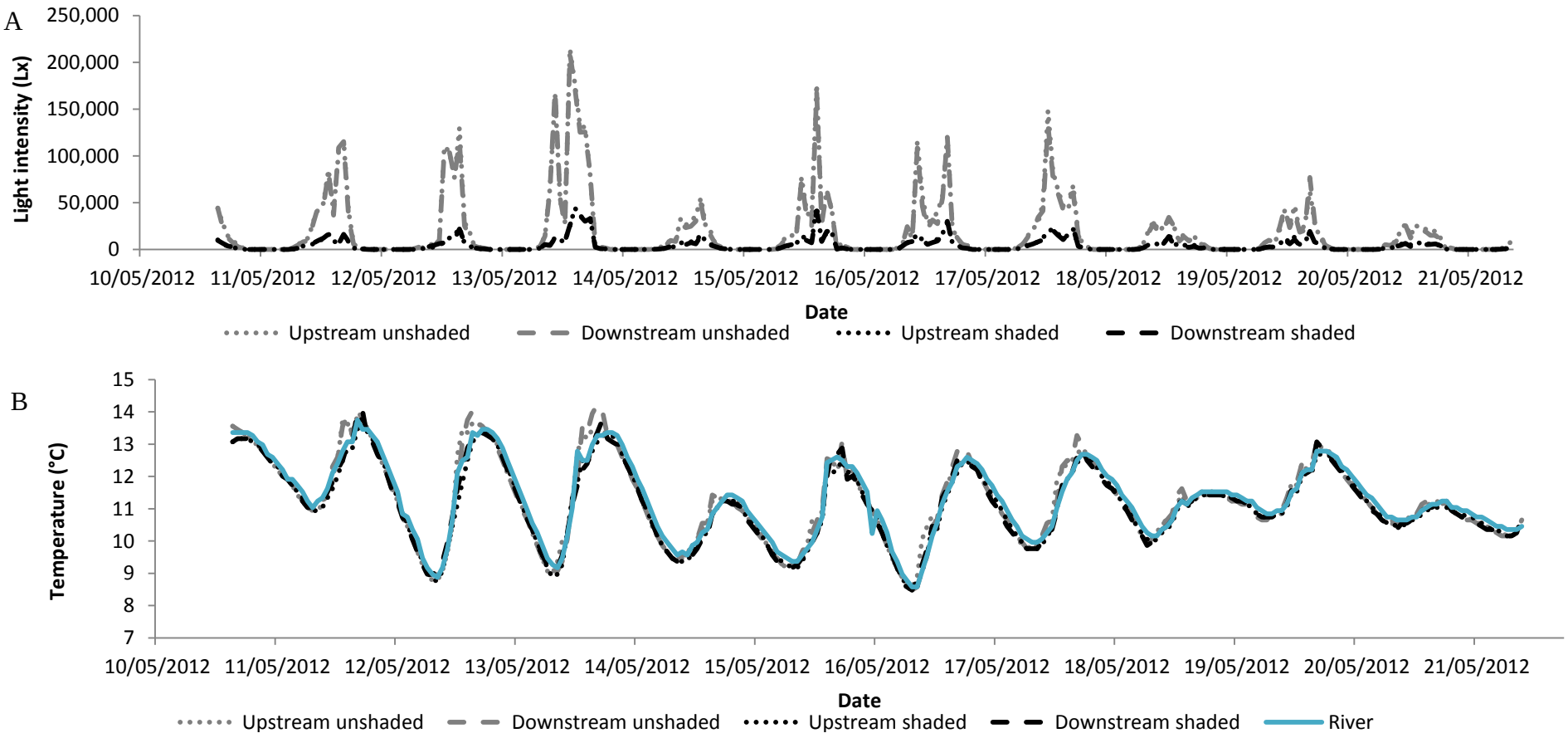


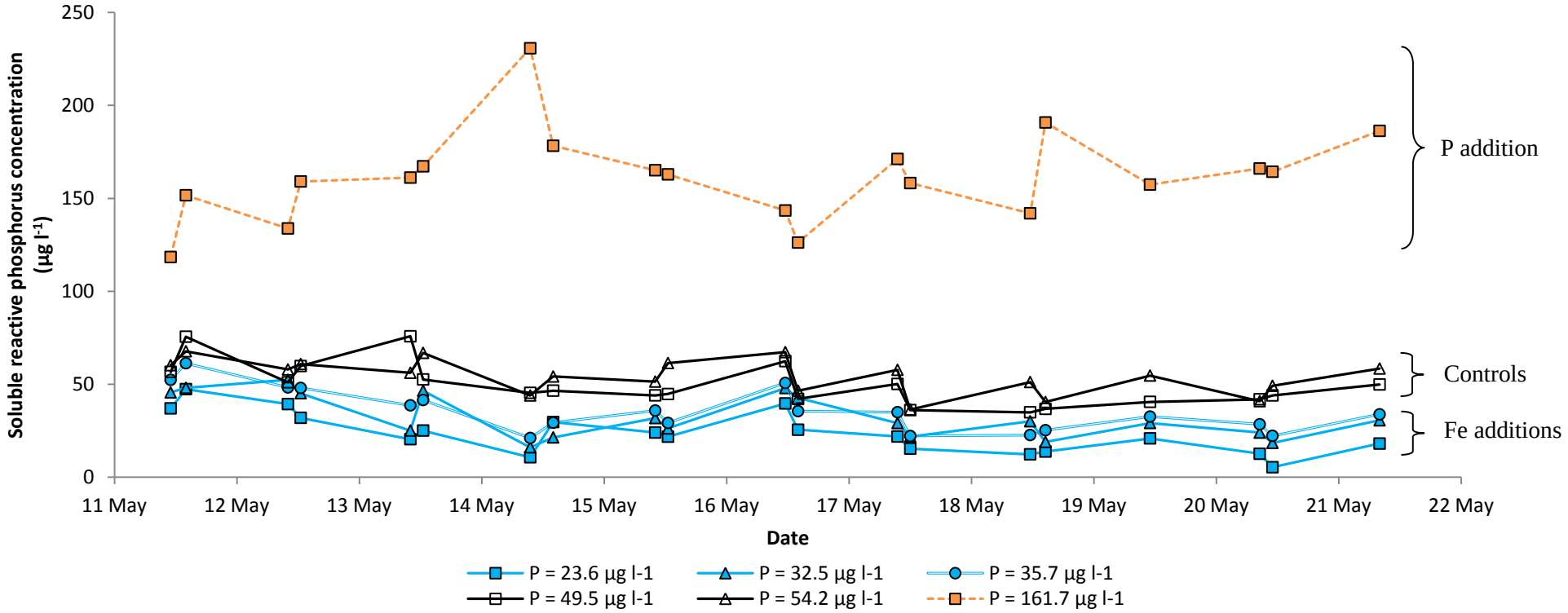
Figure 3.13 (A) The effects of shading in reducing light intensity reaching the ceramic tile artificial substrates in each flume. (B) The effects of shading on temperature in unshaded and shaded sections of flume. The temperature measured in the River Lambourn throughout the 11 day experiment is also shown (blue line).

3.5.2 Flume water chemistry

The SRP concentrations observed in each flume for the duration of the 11 day experiment are shown in Figure 3.14. There was much better control over the phosphorus concentration compared to Experiment 1, especially at the upstream site which had no intermittent phosphorus spikes in the control flumes (Figure 3.14B). The mean control SRP concentrations were higher at the downstream site compared to the upstream site, with larger variation (due to the influence of the STW input, as deduced in Experiment 1). Over the course of the 11 day experiment, SRP downstream ranged from 34.9 to 75.8 µg l^{-1}. The mean concentrations for the two control flumes were 49.8 and 54.2 µg l^{-1}. SRP concentrations in the iron-dosed flumes were reduced by 28, 40 and 52 % to mean concentrations of 35.7, 32.5 and 23.6 µg l^{-1}. The final downstream flume received a phosphorus addition which increased SRP concentration three-fold to an average of 161.7 µg l^{-1} for the duration of the 11 day experiment.

At the upstream site, the SRP concentrations of the iron-dosed flumes were similar to those measured downstream with SRP being reduced by 31, 44 and 49 % to mean concentrations of 30.1, 24.4 and 22.7 µg l^{-1} respectively. The mean of the flumes receiving phosphorus additions upstream were within 15 % of the target concentration, with an average concentration of 146.6 and 156.8 µg l^{-1} across the 11 days. The flume receiving a combined addition of phosphorus and nitrogen had its SRP concentration increased to an average of 122.1 µg l^{-1} and its NO_3-N increased by 15 % from a mean concentration of 6.90 mg l^{-1} (in the river) to a mean concentration of 7.92 mg l^{-1}. Three flumes (one in each set of three) were designated as control flumes. The SRP concentration of these ranged from 25.7 to 57.0 µg l^{-1} with a mean concentration of 42.8, 43.9 and 44.6 µg l^{-1}. These means were higher than expected based on the upstream / downstream simultaneous sampling, due to high rainfall during the experiment leading to an increased surface run off. The simultaneous sampling was conducted in dry weather.

A. Downstream site



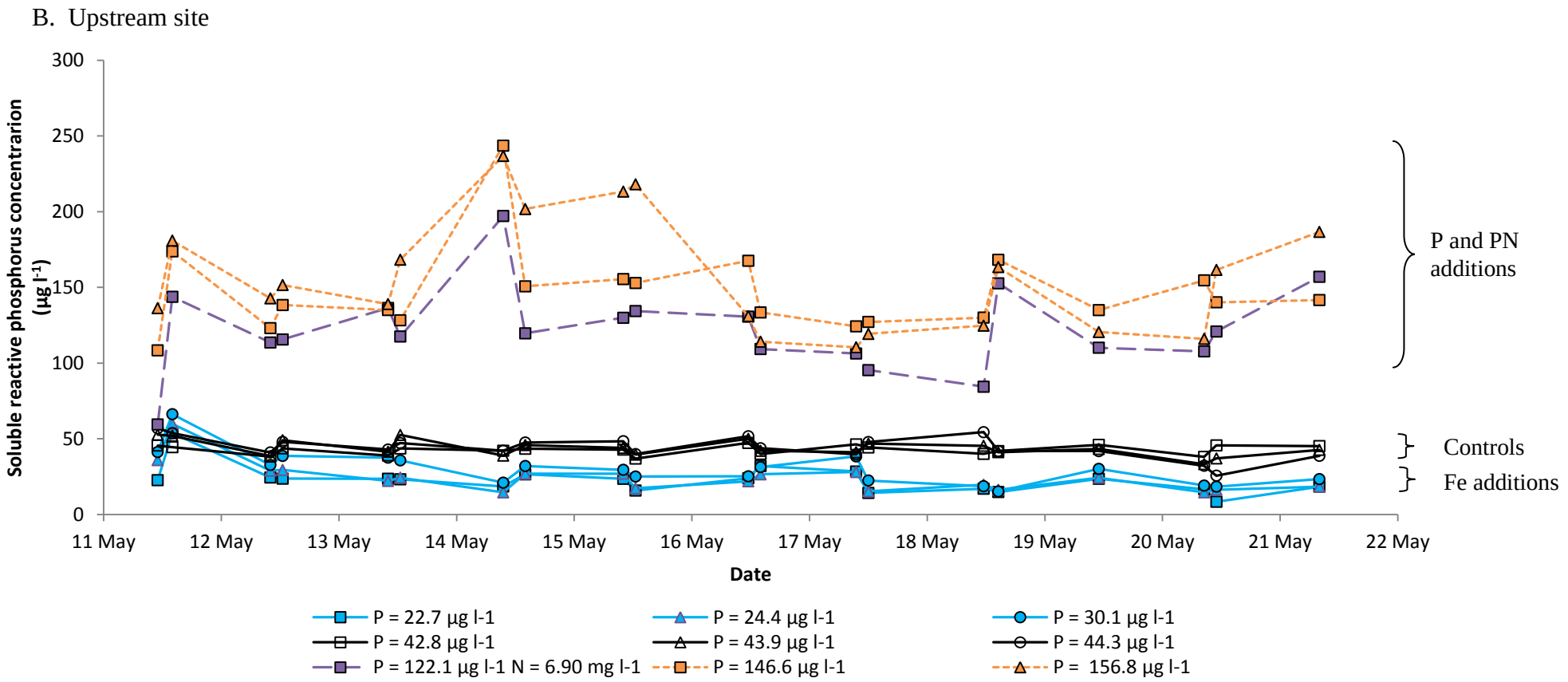


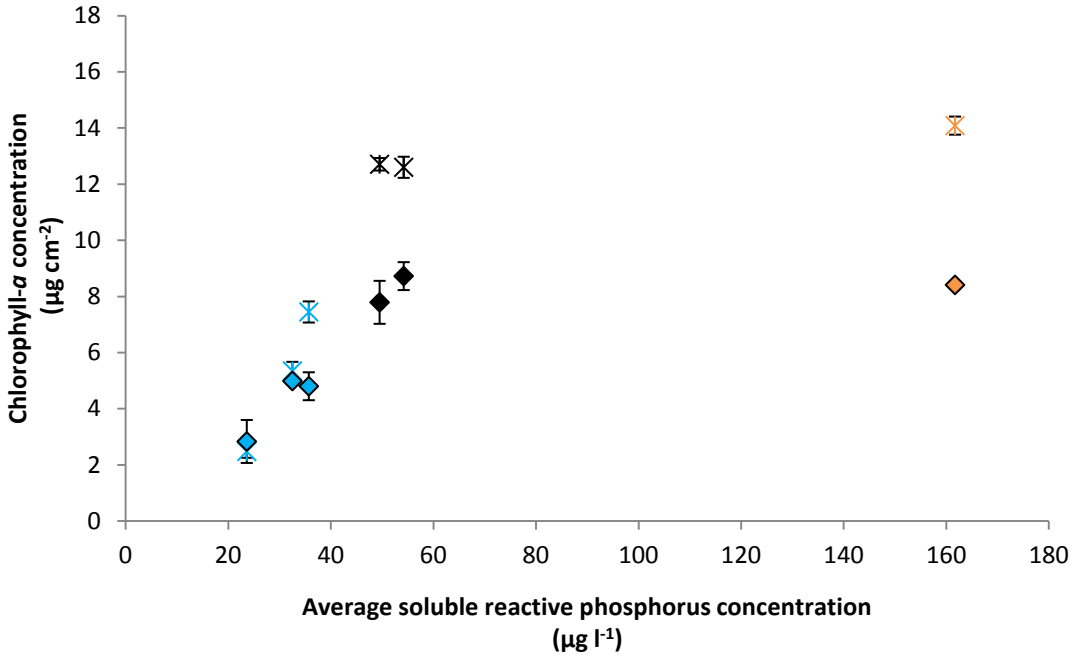
Figure 3.14: Soluble reactive phosphorus (SRP) concentrations in each flume at the sites downstream (A) and upstream (B) of the sewage treatment work input over the course of the 11 day nutrient manipulation experiment. Solid blue line with filled symbol = iron addition (phosphorus reduction), solid black line with open symbol = control (no addition), dashed purple line with filled symbol = phosphorus and nitrogen addition and dotted orange line with filled symbol = phosphorus addition (no nitrogen).

3.5.3 Periphyton biomass response

Both upstream and downstream of the STW input, manipulating nutrient concentrations for 11 days had a significant effect on periphyton biomass regardless of light intensity (Figure 3.15, Figure 3.16 and Table 3.3). The experiment was stopped after 11 days because the periphyton had become increasingly friable and sloughing was imminent in some flumes.

Post-hoc testing (Tukey's HSD test) showed that the addition of phosphorus (either alone or in combination with nitrogen), had no significant effect on chlorophyll-*a* concentration or AFDM at either the upstream or downstream site, showing that periphyton in the River Lambourn were not nutrient limited. At very high SRP concentrations, biomass was actually slightly lower (e.g. Figure 3.15B and Figure 3.16B) which was attributed to some possible sloughing of the periphyton biofilm at the end of the experiment. At higher SRP concentrations, the periphyton colonising the artificial tile substrates appeared to become increasingly filamentous and fragile, and were therefore more easily disturbed and dislodged from the tiles by flow. Upstream of the STW, tiles for Flume 6 (a control flume) were not sampled due to sloughing of the biofilm already occurring while final samples were being collected. The differences in chlorophyll-*a* concentration and AFDM in the remaining control flumes were not significant. This, along with physical variables not being different, provides evidence that the results across different sets of flumes were reproducible. The observed effects when nutrient concentrations were reduced were likely due to nutrient manipulation and not to natural variation in periphyton biomass.

A. Downstream site



B. Upstream site

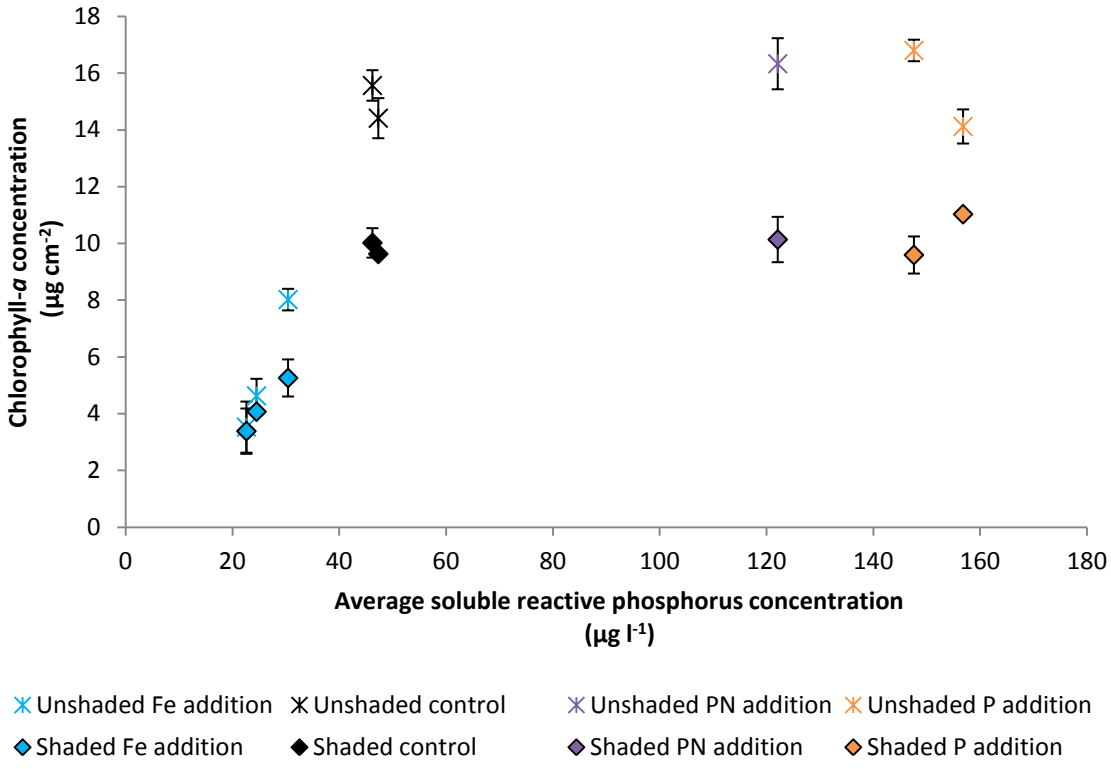
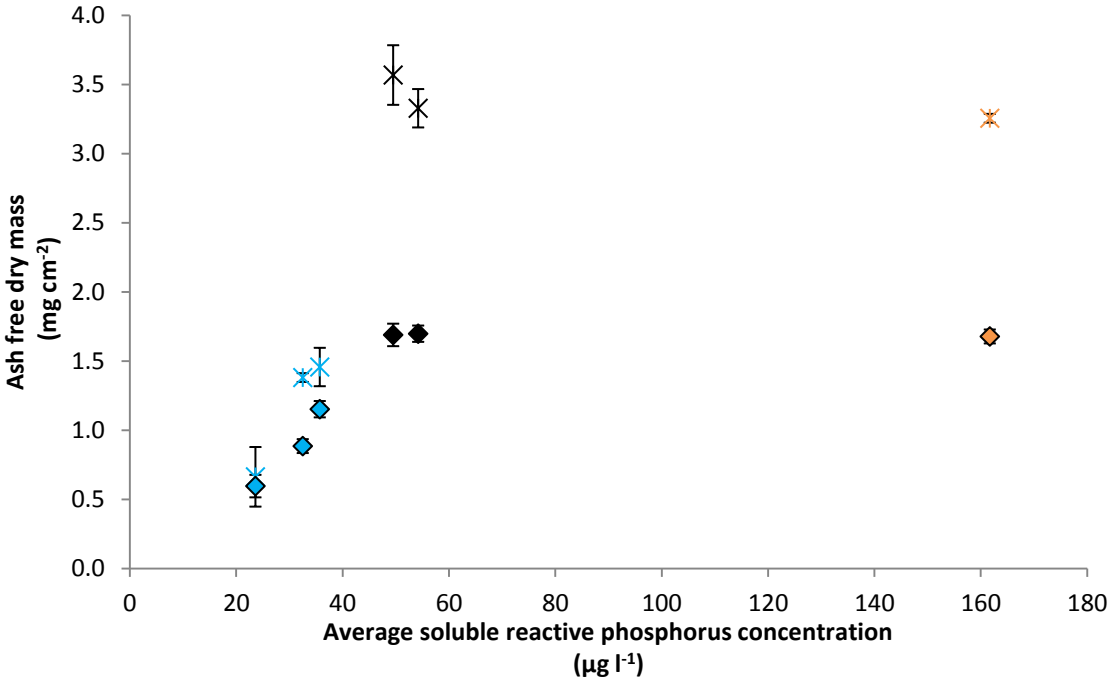


Figure 3.15: Relationship between soluble reactive phosphorus (SRP) concentration chlorophyll-*a* concentration at (A) the downstream and (B) the upstream site on the final day of the flume experiment at both light levels. Data points are mean values based on analysis of three tiles ± one standard error.

A. Downstream site



B. Upstream site

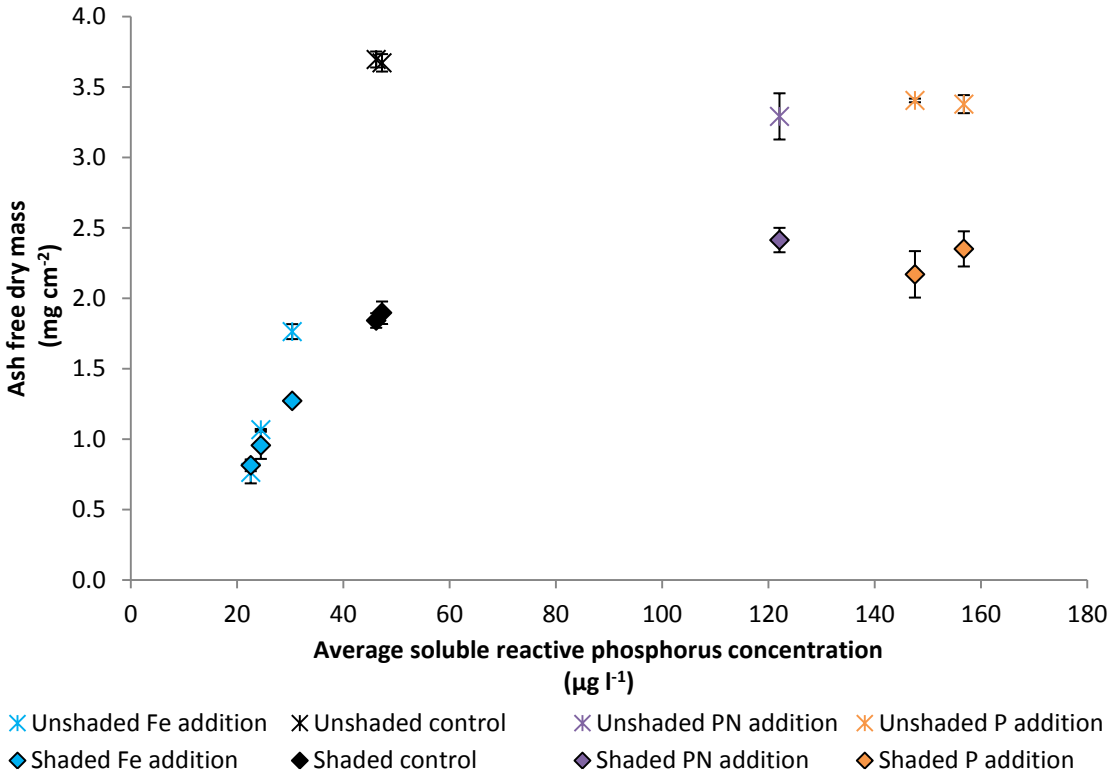


Figure 3.16: Relationship between soluble reactive phosphorus (SRP) concentration ash free dry mass value at (A) the downstream and (B) the upstream site on the final day of the flume experiment at both light levels. Data points are mean values based on analysis of three tiles ± one standard error.

Table 3.3: Statistical significance of manipulating nutrient concentrations within the flume experiment on measures of periphyton biomass.

Variable	Location	Light treatment	One-way ANOVA F statistic	Level of significance (p value)
Chlorophyll-*a*	Upstream	Unshaded	118.12	< 0.001
Chlorophyll-*a*	Upstream	Shaded	46.93	< 0.001
Chlorophyll-*a*	Downstream	Unshaded	205.19	< 0.001
Chlorophyll-*a*	Downstream	Shaded	98.70	< 0.001
AFDM	Upstream	Unshaded	254.17	< 0.001
AFDM	Upstream	Shaded	44.29	<0.001
AFDM	Downstream	Unshaded	130.80	<0.001
AFDM	Downstream	Shaded	61.39	<0.001

Reducing ambient SRP concentration significantly reduced periphyton biomass at both the upstream and downstream sites for both light treatments (Figure 3.15 and Figure 3.16). Reducing ambient SRP concentration by 30 % led to a decrease in chlorophyll-*a* concentration and AFDM of approximately 40 % regardless of light treatment. Reducing ambient SRP concentration further (50 % reduction) reduced chlorophyll-*a* concentration by *ca.* 75 % in the unshaded treatment and 65 % in the shaded treatment. AFDM was reduced by *ca*. 80 % in the unshaded treatments and 60 % in the shaded treatment. Therefore, any reduction in phosphorus concentration in the river will begin to limit periphyton growth due to phosphorus limitation.

In the shaded treatments, light was limiting periphyton growth as indicated by the concentrations of chlorophyll-*a* and AFDM being significantly lower in the shaded treatment (two sample T-test - chlorophyll-*a*: $T = -3.70$, $p < 0.001$; AFDM: $T = -4.67$, $p < 0.001$ – Figure 3.15 and Figure 3.16). For the control and nutrient enrichment treatments, chlorophyll-*a* concentration was *ca.* 40 % less when flumes were shaded, at both the upstream and downstream sites. As SRP concentrations were reduced, the effects of light limitation were less marked (Figure 3.15 and Figure 3.16).

At the lowest SRP concentration upstream of the STW, the difference in chlorophyll-*a* concentration between the unshaded and shaded treatment was reduced to 4 % suggesting that at such low SRP concentrations, light limitation was secondary to

phosphorus limitation. NDS experiments have shown shading to significantly reduce concentrations of chlorophyll-*a* when nutrients were not limiting (Godwin *et al.*, 2009). In addition, co-limitation by light and nutrients has been observed by others working on low nutrient streams both in field (Hill and Knight, 1988) and laboratory based studies (Hill and Fanta, 2008). A similar effect was seen in AFDM. At the lowest SRP concentration, shaded values were lower than unshaded values by 7 % (upstream) and 9 % (downstream). However, at ambient SRP concentrations, shaded AFDM was lower than unshaded AFDM by 49 % at both locations (Figure 3.16).

Based on these total periphyton biomass data, it can be concluded that the River Lambourn is at the phosphorus-limiting threshold of *ca.* 45 µg l^{-1}, as increases in SRP concentration have no effect on periphyton growth rate, and decreases in SRP concentration reduce the growth rate. Previous flume experiments using similar methodology (with river SRP concentrations ranging from 60 to 225 µg l^{-1}) (Bowes *et al.*, 2007, Bowes *et al.*, 2010a, Bowes *et al.*, 2012a) have all concluded that increasing nutrient concentrations has no effect on periphyton biomass accrual rate and that the ambient SRP concentrations were either at or greater than the phosphorus-limiting threshold. This experiment demonstrates that even in relatively clean rivers (by southern England standards) with SRP concentrations of 45 µg l^{-1}, increasing the phosphorus and nitrogen concentration had no effect on periphyton accrual, and therefore nutrients are not limiting.

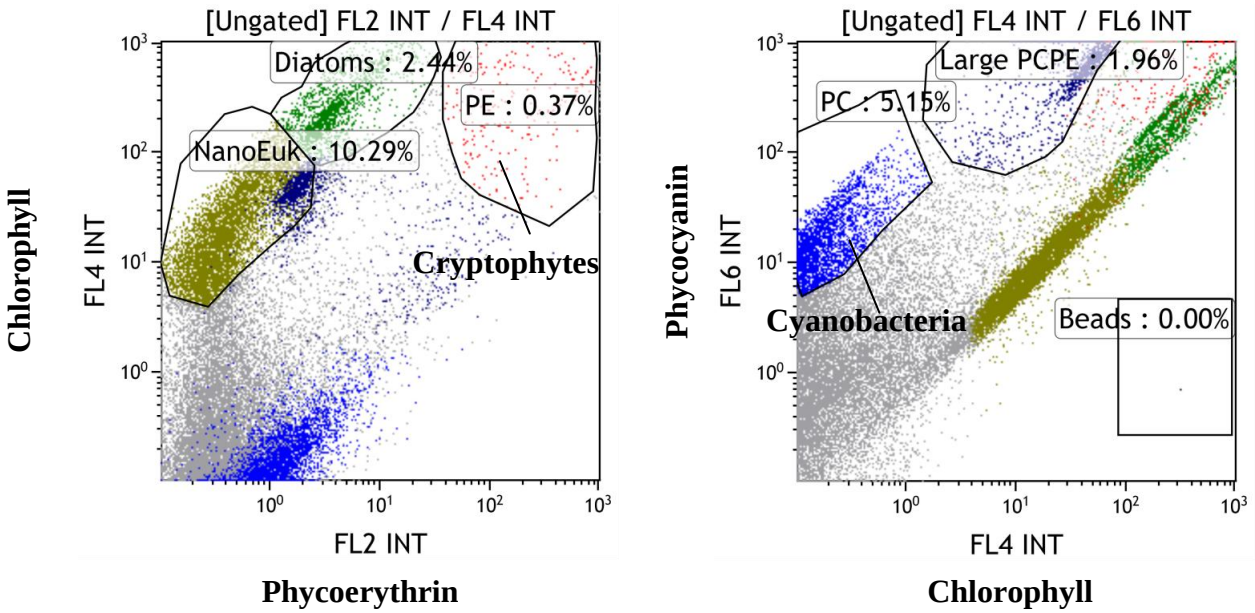
3.5.4 Autotrophic index and flow cytometry

The autotrophic index (AI) of the periphyton biofilm (ratio of AFDM to chlorophyll-*a* concentration) did not show any relationship with changing nutrient concentration regardless of location or light level. This was unexpected, as autotrophs would be expected to proliferate in relation to heterotrophs, in full light. The lack of difference in the AI may be attributed to a large proportion of inorganic sediment (which is non-viable) in the biofilm affecting the AFDM (APHA., 2005).

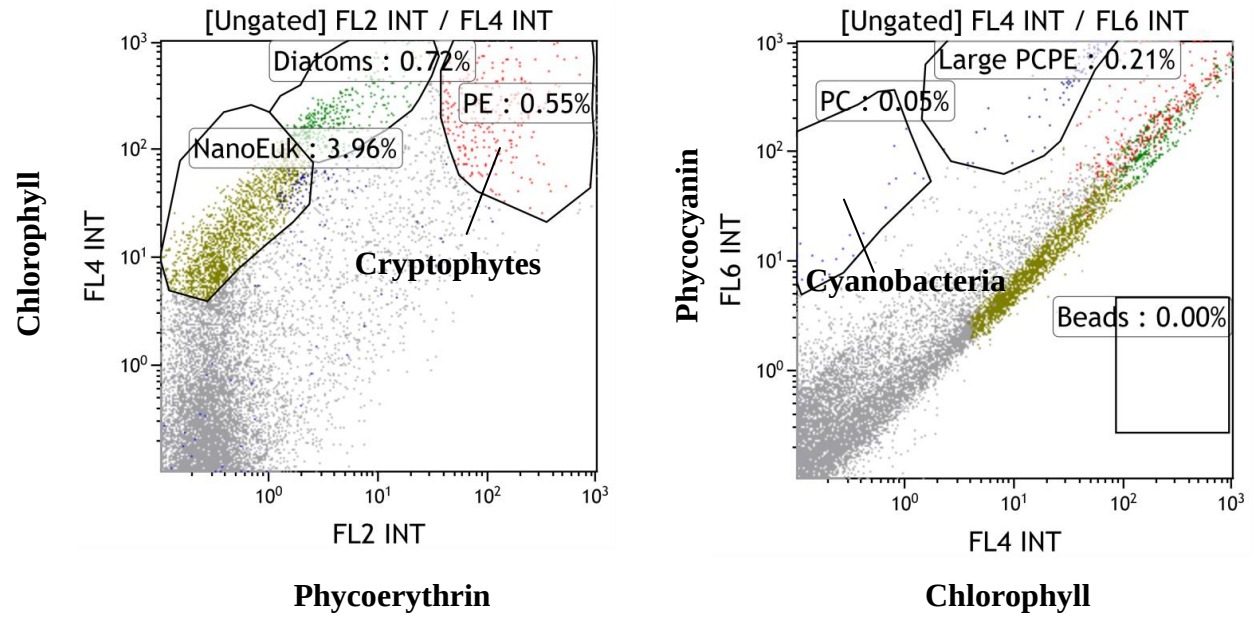
Despite the lack of response in the AI, flow cytometry analysis of the 11 day old biofilms highlighted major shifts in the proportion of functional groups within the periphyton community (Figure 3.17 and Figure 3.18). Figure 3.17 presents raw data

from flow cytometry analysis for the highest and lowest unshaded phosphorus treatments downstream (A) and upstream (B) of Boxford STW input. Each individual dot is representative of a particle that has passed through the flow cytometry detector. The cytograms show black gates characterising the different autotrophic functional groups within the periphyton biofilm community based on size, shape, cell structure and their constituent photosynthetic pigments (Read *et al.*, 2014). Percentages on the figures are the proportion of individual functional groups to the total cell count. Both downstream and upstream of Boxford STW input, reducing phosphorus concentrations resulted in a large decrease in algae and cyanobacterial cell numbers within the biofilms, across all functional groups of the community (Figure 3.17).

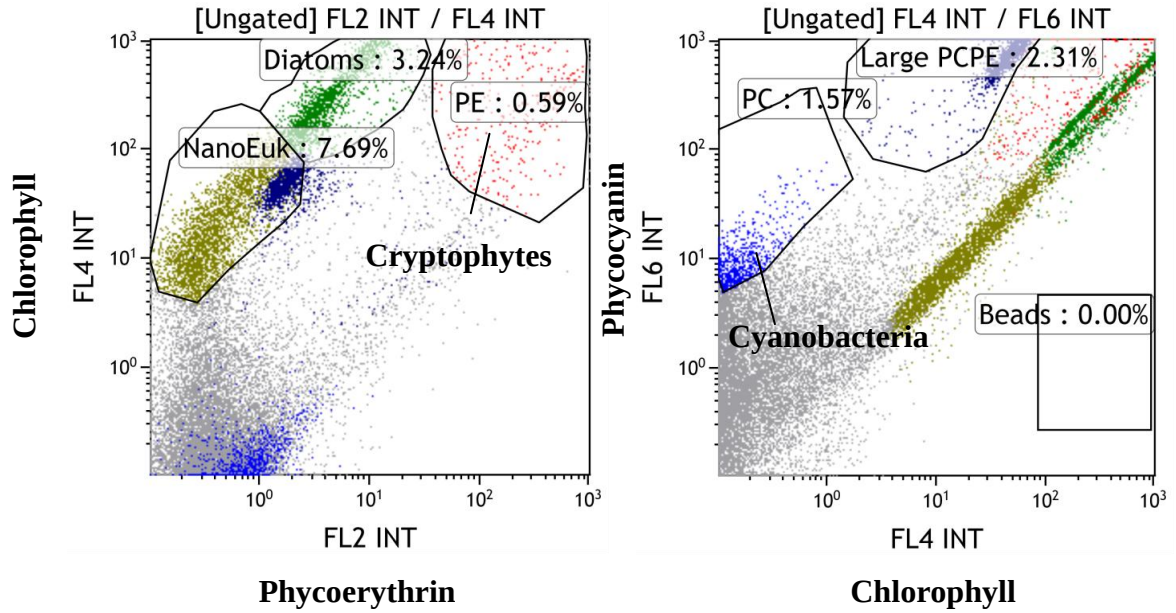
A. Flume 1. Downstream unshaded. SRP = 161.7 µg l^{-1}.



Flume 6. Downstream unshaded. SRP = 23.6 µg l^{-1}.



B. Flume 1. Upstream unshaded. SRP = 156.8 µg l^{-1}.



Flume 9. Upstream unshaded. SRP = 22.7 µg l^{-1}.

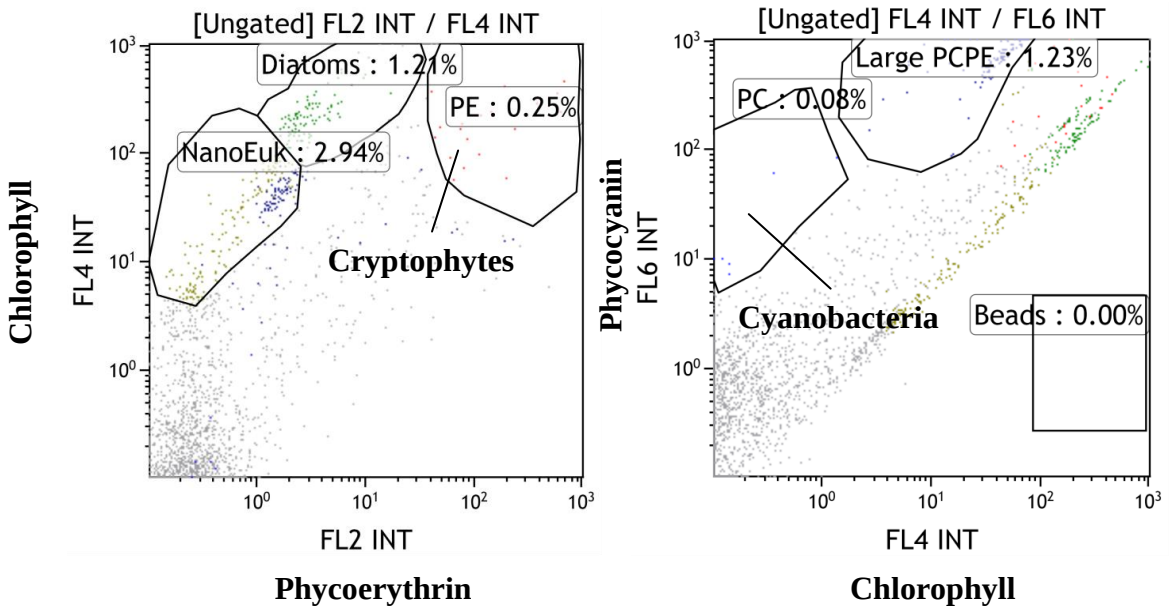


Figure 3.17: Flow cytometry cytograms for the highest and lowest unshaded phosphorus treatment both (A) downstream and (B) upstream of the sewage treatment works input. PC represents phycocyanin containing organisms (i.e. cyanobacteria) and PE represents phycoerythrin containing organisms (i.e. cryptophytes). Percentages represent the proportion of that functional group relative to the total cell count.

Figure 3.18 shows the relative proportions of the different functional groups within the autotrophic community and how these change across the phosphorus concentration gradient at both locations (downstream or upstream of the STW input) and light levels (unshaded and shaded). In all treatments (location and light intensity), nanoeukaryotes and diatoms were more dominant at lower SRP concentrations (Figure 3.18). At the lowest SRP concentration (*ca.* 23 µg l^{-1}) in each light treatment and location, diatoms and nanoeukaryotes consisted of 68 – 80 % of all autotrophic communities present in the biofilm. As SRP concentration increased (to a mean concentration of *ca.* 160 µg l^{-1}), the proportion of diatoms and nanoeukaryotes decreased by approximately half to 32 – 44 % (Figure 3.18). Diatom species have been shown to be particularly adapted to low phosphorus concentrations (Hu and Zhang, 1993). Such species have a competitive advantage over other functional groups due to having a lower half saturation constant for growth and significantly higher maximum nutrient uptake velocities (Holm and Armstrong, 1981). Cryptophytes (phycoerythrin-containing red algae) did not make up a significant proportion of the biofilm (generally less than 5 %) except at the lowest SRP concentrations where they increase to approximately 10 % (Figure 3.18).

Increasing SRP concentration led to a major increase in the proportion of cyanobacteria. In the downstream unshaded flumes for example, cyanobacteria comprised just 8 % of the autotrophic periphyton community when SRP concentration was reduced to 23.6 µg l^{-1}. Yet, in the control treatments (SRP concentration of 49.5 and 54.2 µg l^{-1} and in the phosphorus addition treatment (SRP concentration of 161.7 µg l^{-1}) cyanobacteria comprised *ca.* 50 % of the autotrophic periphyton community. This could have important implications for water quality and water resources / supply, as well as impacting recreation, ecosystem integrity and human and animal health due to the toxicity of cyanobacterial blooms (Downing *et al.*, 2001).

In work undertaken in lakes, cyanobacteria have been implicated to dominate periphyton communities at high phosphorus concentrations due to the ability of certain species to fix atmospheric nitrogen (Schindler, 1977). Increasing SRP concentration can result in a reduced N: P ratio and potential nitrogen limitation of periphyton communities. In this situation, cyanobacteria have a competitive advantage over other autotrophic functional groups within the periphyton biofilm.

Fixing nitrogen eliminates resource limitation, allowing communities to respond to the increased phosphorus concentration without competition from other functional groups (that are nitrogen limited) (Schindler *et al.*, 2008, Vrede *et al.*, 2009). However, the flume experiments presented in this chapter confirm that nitrogen was not limiting periphyton accrual rate in the River Lambourn. This study, therefore, shows that it is the high phosphorus concentration (not low nitrogen concentration or low N: P ratio) of the flumes controlling the periphyton community response (and cyanobacterial dominance). Cyanobacterial cells are large relative to nanoeukaryotes and therefore have a smaller surface area: volume ratio resulting in lowered nutrient uptake at low nutrient concentrations (Lewis, 1976). As phosphorus concentration increases, cyanobacteria are more able to compete with other autotrophic functional groups for nutrients and gain a competitive dominance within the periphyton biofilm.

Increasing abundances of cyanobacteria with phosphorus concentration concurs with recent work in lakes by Downing *et al.* (2001) who concluded that total nutrient concentrations were a better predictor of periphyton community structure compared to nutrient ratios. It also agrees with the work of Ferber *et al.* (2004) who specifically tested the hypothesis of Schindler (1977) and concluded that although nitrogen fixation is important, it was not the only factor affecting cyanobacterial dominance. A mesocosm experiment by Van der Grinten *et al.* (2004) also showed that under nitrogen replete conditions, phosphorus was the dominant factor controlling proportions of diatoms and cyanobacteria with cyanobacteria dominating at higher phosphorus concentrations.

Simultaneously increasing the nitrogen and phosphorus concentration in the River Lambourn had no effect upon biofilm community composition (Figure 3.18). This provides further evidence that phosphorus concentration in the River Lambourn was controlling the periphyton community response, and nitrogen was in excess.

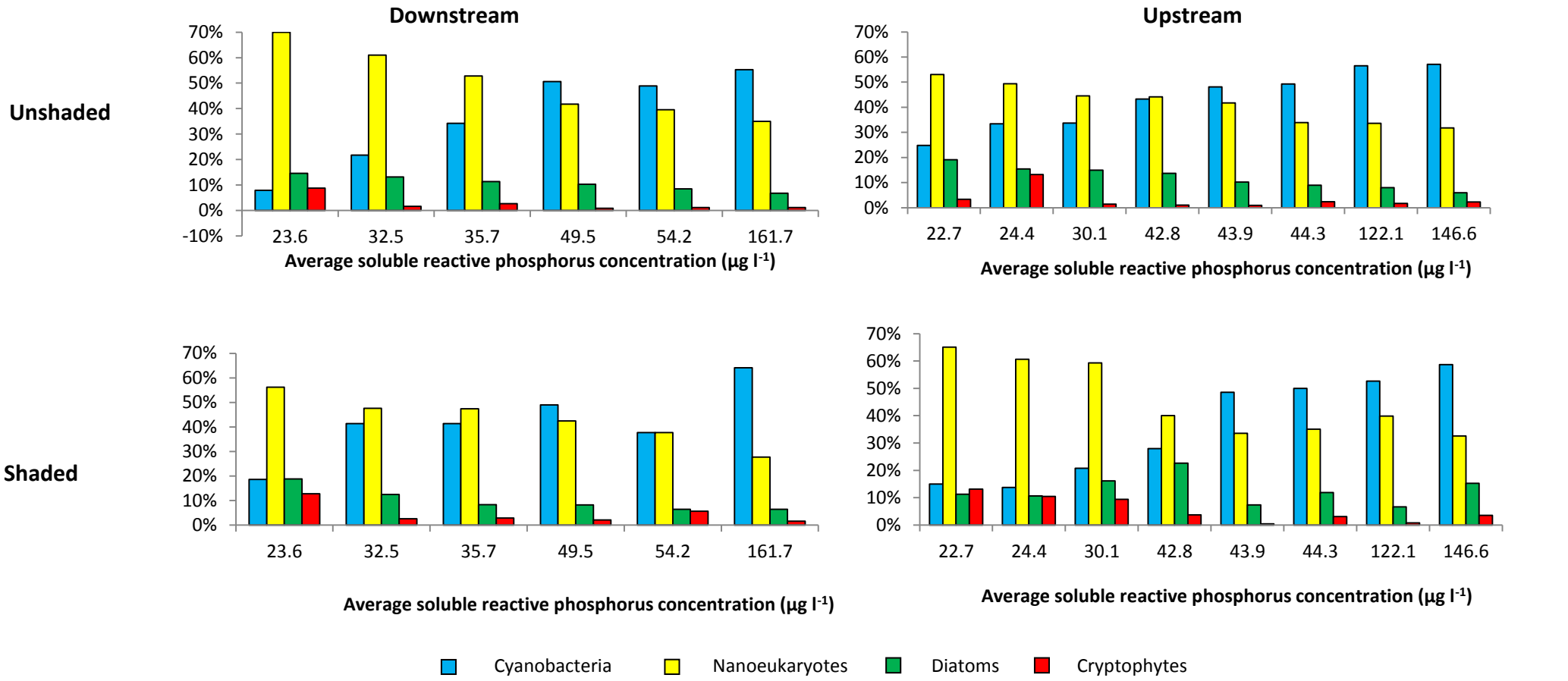


Figure 3.18: Flow cytometry data. Proportion of functional groups within the periphyton biofilm at different nutrient concentrations after the 11 day experiment both downstream (left) and upstream (right) of the sewage treatment work input in both unshaded (top) and shaded (bottom) treatments.

This study has shown that periphyton accrual rate in the River Lambourn is only affected when SRP concentrations are reduced (Figure 3.15 and Figure 3.16). However, community structure (as measured by flow cytometry) is greatly affected by both increases and decreases in SRP concentration (Figure 3.17 and Figure 3.18). This concurs with the work of Bowes *et al.* (2012a) who found that, although the phosphorus-limiting threshold for the River Thames was *ca.* 100 µg l^{-1}, it was only when SRP concentrations were reduced (and maintained) to *ca.* 30 µg l^{-1} that there was a change in diatom community structure and an improvement in the trophic diatom index (TDI). This is the first time flow cytometry has been used to examine periphyton communities in nutrient manipulation experiments and further analysis would need to be undertaken to develop this further. It does, however, suggest that there are two phosphorus thresholds for UK rivers, one which affects periphyton biomass accrual rate (phosphorus-limiting threshold) and a lower threshold which affects the ecology (community structure).

Clearly, cyanobacterial populations need to be controlled if rivers in the UK are to reach the 'good ecological status' required by the WFD, and future climate change scenarios are predicting greater cyanobacterial proliferations due to higher summer temperatures and lower river flows (Whitehead *et al.*, 2013). Work conducted in lakes by Downing *et al.* (2001) suggested 'risk' thresholds for cyanobacterial dominance in lakes, and these are presented in Table 3.4. The results from the present experiment concur with the findings of Downing *et al.* (2001) (Figure 3.18) and suggest phosphorus concentration in the River Lambourn should be kept below 30 µg l^{-1} in order to minimise the dominance of cyanobacterial blooms.

Table 3.4: The risk of cyanobacterial blooms developing (%) at different total phosphorus concentrations (Downing *et al.*, 2001).

Total phosphorus concentration (µg l^{-1})	Risk of cyanobacterial dominance (%)
0 – 30	10
31 – 70	40
~ 100	80

3.5.5 Diatom assemblages

There was no difference in trophic diatom index (TDI) values between the upstream and downstream sites. However, at both locations, increasing nutrient concentrations increased TDI values (Table 3.5). This has previously been found in lake studies where TP has been cited as being responsible for a gradient in diatom species (DeNicola *et al.*, 2004). The TDI of the unshaded control treatments was 44 – 46 (see Appendix B for names, abundance and sensitivity of all species identified and TDI calculations). Based on the expected TDI (Section 2.2.6 and UKTAG (2013b)), this indicated that the river was of high ecological status, both upstream and downstream of the STW input. Of the species identified in the control flumes (ambient phosphorus concentration), approximately half (49 % upstream; 53 % downstream) fell within the TDI sensitivity category of 1 or 2 indicating they favoured very low or low nutrient concentrations (Kelly *et al.*, 2001) with high abundances of *Synedra ulna* (22 % upstream; 19 % downstream) and *Fragilaria capucina* (16% upstream; 11 % downstream).

Table 3.5: Trophic diatom index (TDI) for different nutrient concentrations in the River Lambourn, West Berkshire.

Treatment	Location	SRP concentration ($\mu g\ l^{-1}$)	TDI Unshaded
Iron addition	Downstream	24	39
None (control)	Downstream	55	44
Phosphorus addition	Downstream	162	57
Iron addition	Upstream	23	37
None (control)	Upstream	43	46
Phosphorus addition	Upstream	147	57
Phosphorus and nitrogen addition	Upstream	122	57

Increasing the SRP concentration resulted in an increase in the TDI (Table 3.5). There was a corresponding decrease in the proportion of sensitivity category 1 and 2 species when phosphorus concentrations were greater than 100 $\mu g\ l^{-1}$ (comprised 27 % of total identified upstream and 29 % downstream). *Cocconeis pediculus* (11 % of total species identified up and downstream) and *Cyclotella meneghiniana* (9 % of total downstream, 8 % of total upstream) were the more dominant diatom species at higher SRP concentrations. Both of these species have a sensitivity score of 4

suggesting they favour high concentrations of nutrients (Kelly *et al.*, 2001). However, the diatom community was still representative of a river with high ecological status. The addition of nitrogen in combination with phosphorus did not lead to any further increase in TDI (compared to the phosphorus addition flume) (Table 3.5). This agrees with the results of total periphyton biomass and confirms nutrients were not co-limiting periphyton biomass in the River Lambourn.

Decreasing SRP concentration by 50 % reduced the TDI (Table 3.5). At the same time, there was an increase in species that were tolerant of very low and low nutrient concentrations to 63 % of the total species identified both up and downstream of the STW input. As for the control treatment, *Synedra ulna* (27 % of the total species identified upstream and 24 % downstream) and *Fragilaria capucina* (13 % of the total species identified upstream and 15 % downstream) dominated the diatom flora. This agrees with the findings from the flow cytometry analysis and suggests the phosphorus concentration that leads to a change in community structure is lower than the concentration of the phosphorus-limiting threshold.

3.6 River water quality

A longitudinal survey (described in Section 2.4.1), consisting of seven sites along the main River Lambourn channel, was undertaken at monthly intervals between May 2012 and April 2013 (Figure 3.3; Appendix C). Between May and October 2012, concentrations of both SRP and TP remained relatively stable with concentrations generally increasing downstream (Figure 3.19). Concentrations for June 2012 were slightly higher, potentially due to persistent heavy rainfall leading to increased overland flow and surface run off. Nitrate concentrations in the river were stable over the entire 12 months of the dataset, reflecting the dominance of nitrate-polluted groundwater inputs from the chalk aquifer. Concentrations decreased downstream from an average of 8.93 mg l^{-1}-N at Site 1 to an average of 7.54 mg l^{-1}-N at Site 7. These nitrogen concentrations would not be expected to affect periphyton accrual rate, as they were similar to concentrations measured in the nitrogen treated flumes which resulted in no periphyton biomass response. Phosphorus and nitrate data were not collected from Site 1 and Site 2 during May 2012 as the river was dry due to drought.

Phosphorus concentrations were highest in winter (December 2012 to April 2013) with concentrations in the upstream reaches being particularly high (Figure 3.19), due to the groundwater table being unusually high as a result of heavy rainfall resulting in surcharging of the sewerage network (Environment Agency, 2013, *pers. comm.*). Consequently, untreated sewage was discharged into the river at the village of Lambourn (Site 1 – Figure 3.3A). Due to high flows, this had the greatest impact on phosphorus concentrations at Site 1 and Site 2. Treated sewage input is known to increase SRP concentration of rivers immediately downstream and this was observed in the flume experiment and the longitudinal data as well as in previous studies of water quality in the River Lambourn (Neal *et al.*, 2004b).

The STW at Boxford (between Site 5 and Site 6) appears to have failed in October and November 2012 and April 2013 when downstream SRP concentrations were approximately three times higher than those upstream of the STW (Figure 3.19). As river phosphorus concentrations were at the phosphorus-limiting threshold (as shown by the flume experiments within this chapter), these increases in phosphorus concentration would not be expected to increase periphyton growth rate. It would, however, affect periphyton community structure (with increased cyanobacteria at the expense of diatom species, see Section 3.5.4). The ecological impact would be minimised by the fact that this occurred outside of the main spring-summer growing season. Due to nutrient spiralling (uptake and release of nutrients by algae, macrophytes and sediment) (Newbold *et al.*, 1981, Newbold *et al.*, 1983), the effects of these phosphorus inputs diminished along the river continuum as such that by the time the river water reached the next sampling site (Site 7), increased nutrient concentrations were not detected (Figure 3.19).

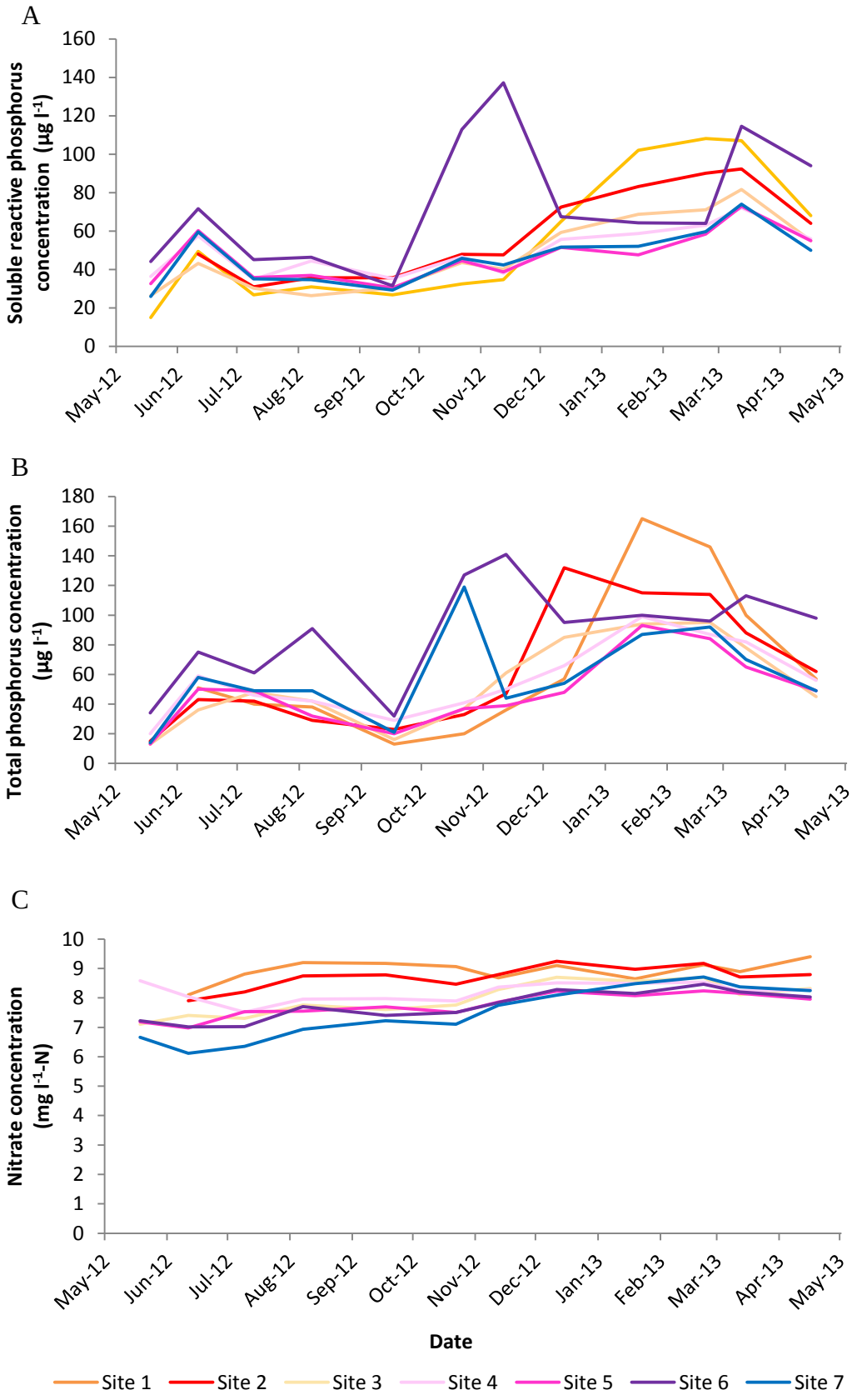


Figure 3.19: Changes in (A) soluble reactive phosphorus, (B) total phosphorus and (C) nitrate concentration in space and time in the River Lambourn.

Between May and November 2012, iron concentrations in the river were between 14.60 and 21.81 µg l^{-1}. These increased to between 42.54 and 72.16 µg l^{-1} downstream of STW between December 2012 and April 2013 as Thames Water increased iron dosing at STW in an attempt to reduce the impact of elevated phosphorus concentrations as a result of sewage leakage.

Chlorophyll-*a* concentration of phytoplankton in the River Lambourn followed a seasonal pattern with values being lowest in winter (November 2012 to January 2013). A spring algal bloom was observed in May 2012 but concentrations were always less than 5.5 µg l^{-1}. This low value reflects the good ecological status of the River Lambourn thus agreeing with TDI calculations from benthic algae. Silicon concentrations mirrored those of chlorophyll-*a*. They were lowest during the May bloom, when diatoms use silicon to produce frustules, as has been observed previously (Neal *et al.*, 2004b). With the exception of June 2012, suspended sediment concentrations also followed a seasonal pattern. Concentrations were higher in winter when rainfall and subsequently river flows were greater and macrophytes were not present. June's sample can be seen as an anomalous result, as for the 24 hours prior to the sample being collected there had been continuous unseasonably heavy rainfall. Suspended sediment concentrations downstream of the STW input at Boxford were consistently higher than for the rest of the river. This may affect river ecology immediately downstream of the STW due to a decrease in light levels.

The flume experiments suggested that phosphorus was limiting to periphyton biomass at SRP concentrations below 45 µg l^{-1} and affected community structure once concentrations below 30 µg l^{-1} were achieved. Analysis of all longitudinal survey data (both in space and time) suggests SRP concentrations could potentially limit periphyton biomass at Lambourn, Great Shefford and Shaw during September 2012 and at Great Shefford and Shaw during the spring algal bloom (May 2012), as SRP concentrations measured were below 40 µg l^{-1}.

As phosphorus concentrations increased above *ca.* 50 µg l^{-1}, the community composition of periphyton biofilm in the flume experiment changed to those dominated by cyanobacteria. In the river, SRP concentrations were over 50 µg l^{-1} at the downstream sites (Sites 4 – 7) in June 2012. This can be attributed to heavy

rainfall in the 24 hours prior to sampling. The higher SRP concentrations would potentially affect the community composition of periphyton biofilms, and encourage cyanobacterial growth during an ecologically sensitive time of the year. In addition, on all sampling occasions between December 2012 and the end of sampling in April 2013, all sites along the entire length of the River Lambourn had SRP concentrations greater than 50 µg l^{-1} (Figure 3.19).

Nutrient concentrations within the River Lambourn were higher after periods of heavy rainfall. This was indicative of large quantities of diffuse nutrient pollution being washed into the river from surrounding agricultural fields as a result of surface run off and subsurface flow. Nutrient concentrations within the river could be further managed by the installation of riparian buffer strips along the channel margins (Stutter *et al.*, 2012). As well as reducing diffuse nutrient inputs to streams by absorbing nutrients, riparian buffer strips would have a number of other advantages for the wider stream community including habitat provision for invertebrates and mammals, bank stabilisation (reduced soil erosion, another phosphorus source) and increasing connectivity between the terrestrial and aquatic environments.

3.7 Conclusions

Results from these experiments suggest that nutrient (phosphorus and nitrogen) concentrations currently observed in the River Lambourn are not limiting total periphyton growth and that the ambient SRP concentration of the river (45 µg l^{-1}) is at the phosphorus-limiting threshold. Increasing SRP concentration three-fold to a mean of *ca.* 160 µg l^{-1} did not have any significant affect on chlorophyll-*a* concentration or AFDM. When SRP concentration was decreased, there was a significant corresponding decrease in periphyton biomass. A 50 % reduction in SRP concentration resulted in a 75 and 65 % reduction in chlorophyll-*a* concentration for unshaded and shaded artificial substrates, respectively, and a 80 and 60 % reduction in AFDM (unshaded and shaded). Positioning the flumes upstream and downstream of Boxford STW in Experiment 2 did not show any significant difference in periphyton biomass or community composition of the control flumes, suggesting an average difference in SRP concentration of 10 µg l^{-1} was insufficient to impact on

the periphyton community. Both sites confirmed that a SRP concentration of *ca.* 45 µg l^{-1} was the phosphorus-limiting threshold.

Although total periphyton biomass did not change with increasing phosphorus concentration, the functional groups (community composition) within the biofilm and the tolerance of diatom species did. At lower SRP concentrations, nanoeukaryotes and diatoms dominated the periphyton biofilm. As SRP increased, cyanobacteria become the dominant functional group. At the same time, there was a shift in diatom species from those least tolerant to those most tolerant of nutrients as SRP increased.

Light was also important in controlling periphyton accrual. Shaded tile substrates accrued significantly less periphyton than unshaded tiles at each nutrient concentration, suggesting light was limiting growth under these shaded conditions. Across control and nutrient enrichment treatments, shaded substrates accrued *ca.* 40 % less biofilm. The biofilm that grew in the shaded treatments was of higher nutritional quality to grazing invertebrates and had the potential for higher food chain efficiency compared to unshaded flumes. The effect of light on total biomass was reduced at the lowest nutrient concentrations, suggesting that light limitation was secondary to phosphorus limitation at SRP concentrations less than 25 µg l^{-1}. However, in the unshaded treatments, light played a greater role in reducing food quality at lower nutrient concentrations.

The fact that the river is at the phosphorus-limiting threshold suggests further decreasing SRP concentration would be likely to have a positive ecological effect on the river, by reducing periphyton growth rate, thereby encouraging a macrophyte-dominated river ecosystem (Hilton *et al.*, 2006). The microbial community composition would also be shifted towards having a lower percentage of cyanobacteria and to low-nutrient favouring diatom species, thereby improving the TDI score and ecological status. The larger STW discharging into the river are already subject to phosphorus-stripping, meaning further reductions in SRP concentration would be difficult and expensive to achieve. In addition to reducing phosphorus, these experiments suggest that the addition of riparian shading at the channel margins would also have a positive ecological effect. Shading could prove to be a cost-effective way of reducing periphyton biomass.

In terms of managing the fragile chalk stream environment in the future, a combination of further SRP reductions and riparian shading should be considered. These two factors would result in large reductions in periphyton biomass (from *ca.* 14 to 2 µg cm^{-2}) (Figure 3.15 and Figure 3.16) and cyanobacteria populations (from 40 to ~ 16 %) (Figure 3.18) as well as an improvement in the nutritional quality of periphyton (Figure 3.11). This work has important policy implications, in terms of the UK meeting the targets of the WFD since, even in one of the cleanest rivers in southern England, phosphorus concentrations are still at the threshold and not truly limiting to periphyton growth.

Chapter 4: Sequential co-limitation of periphyton biofilms in the River Rede, Northumberland.

4.1 Introduction

Reducing phosphorus loading to rivers is still seen as the main mitigation measure to improve aquatic ecology (Gold and Sims, 2005), as phosphorus is widely assumed to be the limiting nutrient in most rivers, thereby constraining primary production (Chapter One). Even in relatively pristine, low nutrient rivers, the reduction of phosphorus inputs remains one of the primary mitigation options to improve ecological status. An example of this is the River Rede, Northumberland. Its water quality is classified as Very Good by the Environment Agency, with dissolved oxygen concentration being over 90 %, nitrate concentration of less than 0.5 mg l^{-1} (Baker and Inverarity, 2004), and SRP concentrations of less than 20 µg l^{-1}. The river is classified as being oligotrophic (Dodds *et al.*, 1998) and is of national and international importance, as it is one of the few remaining sites in the UK where the endangered freshwater pearl mussel (*Margaritifera margaritifera*) can be found.

4.1.1 Freshwater pearl mussels

In Britain, freshwater pearl mussels are protected under the Wildlife and Countryside Act (1981) and have a specific Species Action Plan under the UK Biodiversity Habitat Action Plan in which they are classified as a priority species. Despite this, Bauer (1988) estimated a decline of over 90 % of pearl mussels across populations in central Europe. Pearl mussels are a long-lived species commonly reaching ages of over 100 years (Bauer, 1988). The demographics of the population in the River Rede suggest a failure in recruitment over the last 30 years and an aging population with no reproduction (Environment Agency, 2011, *pers. comm.*).

In addition to pearl hunting, which is now illegal, a number of other factors have been implicated in the decline in pearl mussel populations and lack of breeding success across the UK in recent years. The main issue is thought to be declining water quality and increasing eutrophication caused by excess nutrients and organic

pollution (Bauer, 1988, Frank and Gerstmann, 2007). River engineering activities (Cosgrove and Hastie, 2001), large-scale flooding (Hastie *et al.*, 2001), increased turbidity (Österling *et al.*, 2008) and sedimentation (Box and Mossa, 1999) have also been attributed to the species decline and failure in recruitment.

4.1.2 Experimental aims and hypotheses

Previous flume experiments (Bowes *et al.*, 2007, Bowes *et al.*, 2010. Bowes *et al.*, 2012a, Chapter Three of this thesis) have found ambient SRP concentrations to either be at or above the phosphorus-limiting threshold. The previous chapter (Chapter Three) concluded that ambient SRP concentrations of 45 µg l^{-1} were still not limiting periphyton growth. Previous research has indicated that phosphorus concentration must be reduced to below 30 µg l^{-1} in order to observe an ecological effect (Dodds *et al.*, 2002, Chambers *et al.*, 2012).

The present experiment applied the flume mesocosm methodology (described in Sections 2.1 to 2.3) to a river that would be expected to be strongly phosphorus limited, the oligotrophic River Rede (N: P ratio of 50: 1). This experiment aimed to examine how periphyton growth rate responded to increasing SRP concentrations, allowing the phosphorus-limiting threshold to be quantified. Evidence from recent studies have also showed that phosphorus and nitrogen often co-limit growth in oligotrophic systems (Elser *et al.*, 2007), and so the effects of nitrogen enrichment and a combination of phosphorus and nitrogen enrichment were also examined.

This study aimed to identify whether the present phosphorus and nitrogen concentrations in the River Rede have an impact on primary production and ecological status (potentially affecting freshwater pearl mussel populations), and thereby establish whether the nutrient mitigation strategies presently employed in the catchment have a beneficial effect on ecological status. The specific hypotheses tested (at a significance level of 0.05) were:

1. **H_1 -** SRP concentrations are limiting periphyton growth and accrual in the oligotrophic River Rede. Therefore, increasing concentration will result in a significant increase in periphyton biomass (as measured by chlorophyll-*a* concentration and ash free dry mass (AFDM)).

H_0 - SRP concentrations are not limiting periphyton communities in the River Rede. Therefore, the relationship between SRP and periphyton biomass is not significant**.**

2. **H_1** - Increasing SRP concentration will significantly affect diatom communities in the River Rede. There will be an increasing proportion of nutrient-tolerant species with increasing SRP and a corresponding (significant) increase in the TDI.

   H_0 - Increasing SRP concentration will have no significant affect on diatom communities in the River Rede.

4.1.3 Catchment description and study site

The River Rede is a 58 km long tributary of the North Tyne River, rising within the Cheviot Hills, north-east England and entering the North Tyne at the village of Redesmouth (Figure 4.1). The upland catchment has a total area of 343.8 km^2 and is underlain by Carboniferous limestone and sandstone formations, overlain by superficial deposits of boulder clay, alluvium and peat (Lawrence *et al.*, 2007, Marsh and Hannaford, 2008). Mean annual rainfall in the catchment is 1026 mm and the river has a particularly flashy nature with a base flow index of 0.33 (Marsh and Hannaford, 2008).

Although the area has a low human density (less than 1 % of the catchment is classified as urban), the upper reaches of the river are heavily modified due to impoundment by Catcleugh Reservoir (built 1905), which covers 40 km^2 (11 %) of the catchment, maintaining low flows of 0.158 m^3 s^{-1} (Petts *et al.*, 1993). The main land uses within the catchment are agricultural grazing (39 %) and coniferous forestry (31 %) (Fuller *et al.*, 2002).

The portable in-stream flume mesocosms were installed in the River Rede near the village of Otterburn (grid reference NY 890 926). Potential small point source nutrient inputs to the river upstream of the study site arise from a minor STW located at Byrness (population estimate of 168) and a water treatment works at Rochester. Diffuse nutrient inputs arise from individual septic tanks, and agricultural and

forestry activities. Otterburn sewage treatment works (population estimate of 550) was 50 m downstream of the study site and there are two further STW discharging treated final effluent into the lower river at West Woodburn (population estimate of 128) and Redesmouth (population estimate of 45) (Figure 4.1).

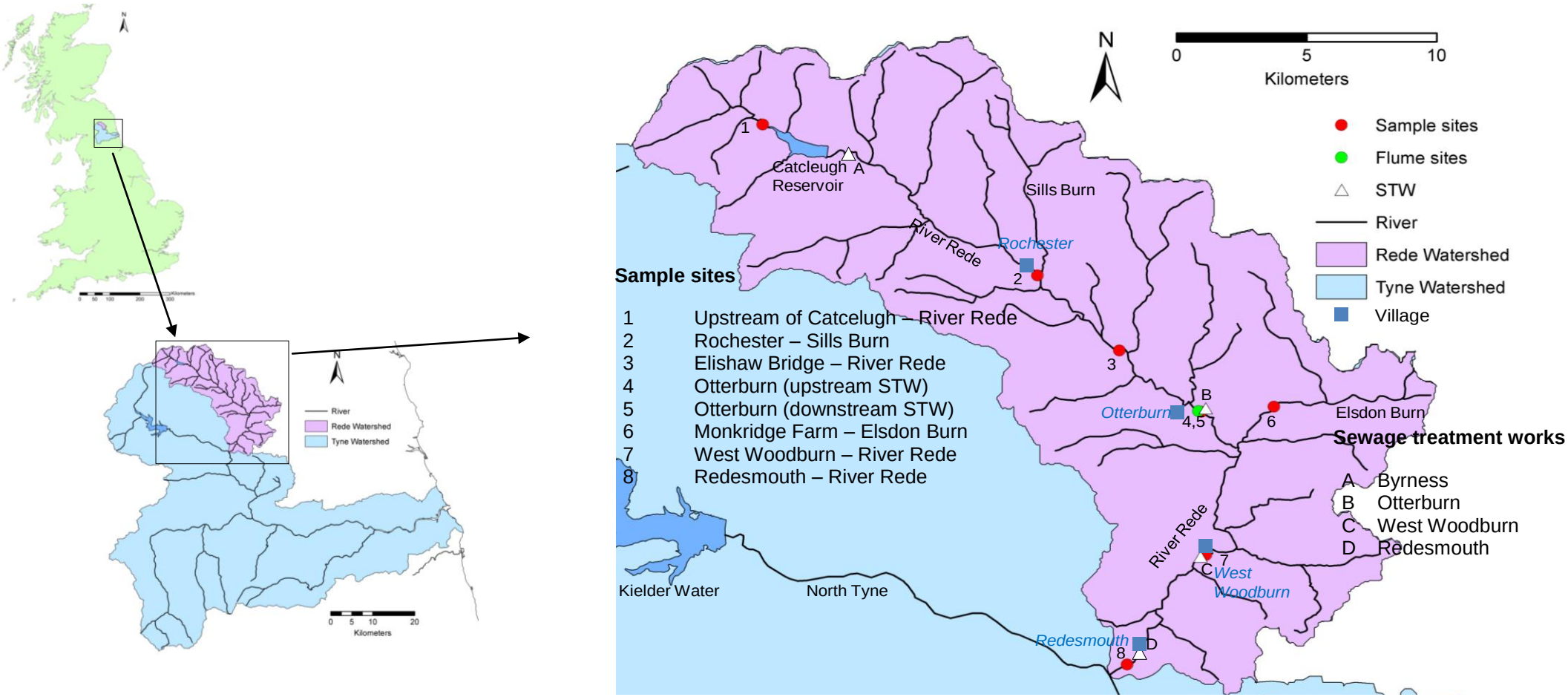


Figure 4.1: Map of the River Rede catchment, Northumberland showing the location of the flume experiment at Otterburn. Numbers denote river sampling sites as part of a longitudinal survey. Letters denote sewage treatment works.

4.2 Experiment-specific methodology

Twelve in-stream flume mesocosms were installed along a 40 m straight, unshaded section of the River Rede at Otterburn in June 2011 (see Section 2.1 for further details). Flumes were secured in place using scaffolding poles pile-driven into the river bed (Figure 4.2). The flow velocity gates were set so the water velocity within each flume at the start of the experiment was 0.12 m s^{-1}. In order to relate the results from the flume experiments to water quality along the River Rede, a longitudinal water quality survey of the River Rede, two of its major tributaries and the final sewage effluent from Otterburn STW was conducted (Figure 4.1**)**. Samples were collected from the main flow of the river on the 1st July 2011 and analysed according to the methods described in Section 2.4.

Figure 4.2: Photograph showing the flume experimental set-up in the River Rede at the village of Otterburn. The blue arrow represents direction of river flow.

4.2.1 Experimental treatments

A range of nutrient concentrations were simultaneously produced in the 12 flumes by the addition of concentrated nutrient solutions to the incoming river water (Table

4.1). To identify the phosphorus-limiting threshold, five flumes received different levels of phosphorus additions. Another flume was dosed with an iron solution, with the aim to reduce the river's SRP concentration, using a phosphorus-stripping methodology (Bowes *et al*., 2007, Bowes *et al*., 2010, Bowes *et al*., 2012a).

To investigate whether periphyton communities were limited or co-limited by nitrogen, one flume received nitrogen addition and one received a combined phosphorus and nitrogen addition. One flume in each set of three received no chemical addition, thereby acting as a control, with unmodified river water flowing through it for the duration of the experiment. The choice of nutrient treatment in each flume and position of controls within each set of three flumes were randomly assigned.

Table 4.1: Target nutrient concentrations during the experiment on the River Rede from 24th June to 3rd July 2011. Increases and decreases were based on an ambient SRP concentration of 15 µg l^{-1} and NO_3-N concentration of 0.70 mg l^{-1}.

Flume number	Nutrient treatment	Target SRP concentration	Target nitrate concentration
1	P addition	4x increase	N/A
2	None (control)	N/A	N/A
3	P addition	6x increase	N/A
4	None (control)	N/A	N/A
5	P addition	3x increase	N/A
6	P addition	2x increase	N/A
7	Fe addition	< 10 µg l^{-1}	N/A
8	None (control)	N/A	N/A
9	P addition	10x increase	N/A
10	P and N addition	10x increase	2x increase
11	N addition	N/A	2x increase
12	None (control)	N/A	N/A

Light intensity and temperature were recorded hourly in each of the four sets of flumes throughout the nine day experiment using HOBO data loggers (the same as those used in the River Lambourn experiment). The exact flume in which the logger was placed within the set of three was randomly assigned. At 15:30 on 28th June 2011, the logger from Flume 10 was removed and placed at a similar depth in the

main river channel to record stream water temperature. The experiment ran for nine days from 24th June to 3rd July 2011.

4.3 Results and discussion

4.3.1 Light intensity and temperature

Figure 4.3A shows the light intensity measured in four of the flumes throughout the nine day experiment. Tiles were in daylight for 17 hours per day between 5:00 am and 10:00 pm. Average light intensities during daylight hours in Flume 2, 6 and 8 were 34 859, 34 828 and 34 192 Lx respectively. The average light intensity in Flume 10 was 30 113 Lx. Light was not measured in Flume 10 after 15:00 on 28th June 2011, when the HOBO logger was moved into the river channel. As Figure 4.3B shows, there was little difference in temperature between the sets of flumes and the river channel. The average temperatures recorded in the flumes over the course of the experiment were 16.96 ± 5.66, 16.99 ± 5.82, 16.89 ± 4.97 and 16.96 ± 5.18 °C for Flumes 2, 6, 8 and 10 respectively. The river temperature was slightly lower than that of the flumes with an average temperature of 16.48 ± 3.09 °C

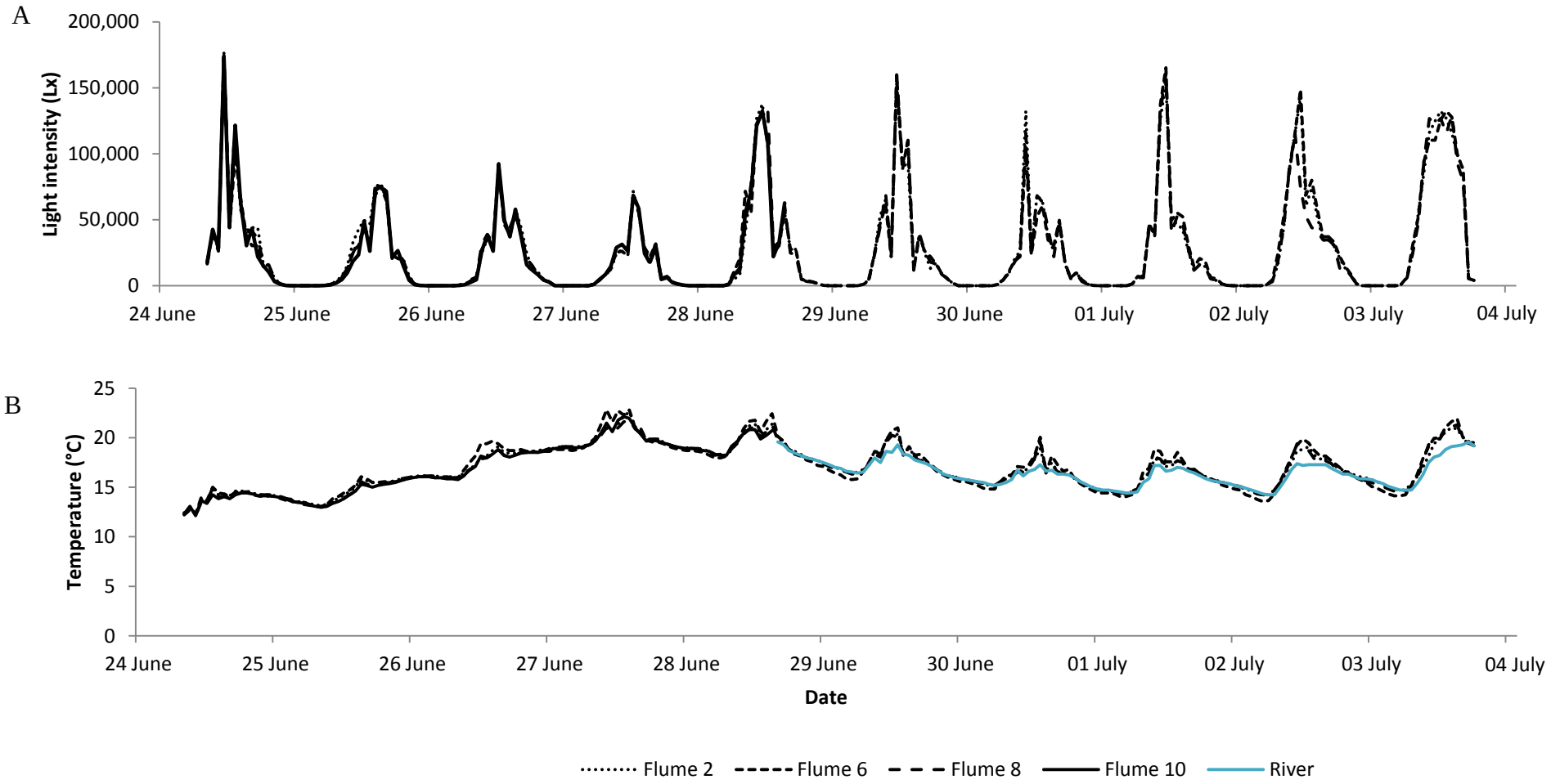


Figure 4.3: (A) Light intensity measured in four of the flumes at hourly intervals during the experiment. (B) Water temperature measured in four of the flumes and the River Rede at hourly intervals during the experiment.

4.3.2 Flume water chemistry

The SRP and nitrate concentrations produced in each flume for the duration of the nine day experiment are shown in Figure 4.4 and Figure 4.5, with average concentrations given in Table 4.2. The control flumes had average SRP concentrations between 14 and 17 µg l^{-1} during the nine day experiment. The average nitrate-N concentration in the control flumes during the experiment was 0.76 mg l^{-1}. Nitrogen concentration was increased at the start of the experiment to 1.30 and 1.37 mg l^{-1} in two of the flumes, so that nitrogen concentrations were increased by approximately 80 % (Table 4.2). The flume that had its nitrogen concentration increased to 1.30 mg l^{-1} simultaneously had its SRP concentration increased to 134 µg l^{-1}. Phosphorus was added to five flumes, successfully producing a continuum of SRP concentrations ranging from 30 to 130 µg l^{-1} (Figure 4.4). The resulting N: P ratios ranged from 45 to 54: 1 in the control flumes, to 6: 1 in the flume receiving the largest P addition. The flume receiving only N addition had an N: P ratio of 91: 1, while the flume receiving a combination of P and N had a ratio of 10: 1 (Table 4.2).

Unlike in previous experiments (Bowes *et al.*, 2007, Bowes *et al.*, 2010a, Bowes *et al.*, 2012a), the addition of iron sulphate to Flume 7 failed to reduce SRP concentrations (Figure 4.4). On 27th June 2011 (day three of the experiment), the iron stock solution was changed from iron sulphate to a concentrated solution of iron (III) chloride ($FeCl_3$). This also failed to strip phosphorus from the river water in the flumes. Consequently on 29th June 2011 (day five), the iron sulphate was reintroduced to the flume in addition to the iron chloride. When this failed to reduced SRP concentration (SRP in iron dosed flume = 7.72 µg l^{-1}, SRP in control flume = 7.46 µg l^{-1}), iron dosing was terminated and results from tiles in Flume 7 were excluded from further analysis. The failure of iron to strip phosphorus in the River Rede was unexpected and attributed to chemical interferences in this highly-organic, peaty river water. Furthermore, the ambient iron concentration in the River Rede was much higher than in river systems where iron had been used successfully (640 µg l^{-1} (see Table 4.4) compared to a concentration of 57 µg l^{-1} in the River Lambourn).

Table 4.2: Average nutrient concentration, percentage increase in nutrient concentrations and N: P ratios measured in the flumes across the nine day experiment.

	Average nutrient concentration		Percentage increase in nutrient concentration		
Nutrient treatment	SRP ($\mu g\ l^{-1}$)	NO_3 - N ($mg\ l^{-1}$)	P (%)	N (%)	N:P
P addition	58	(0.76)	263	N/A	13:1
Control	16	(0.76)	N/A	N/A	48:1
P addition	87	(0.76)	444	N/A	8:1
Control	15	(0.76)	N/A	N/A	51:1
P addition	39	(0.76)	160	N/A	19:1
P addition	30	(0.76)	100	N/A	25:1
Control	14	(0.76)	N/A	N/A	54:1
P addition	130	(0.76)	829	N/A	6:1
P and N addition	134	1.30	88	71	10:1
N addition	15	1.37	N/A	80	91:1
Control	17	0.76	N/A	N/A	45:1

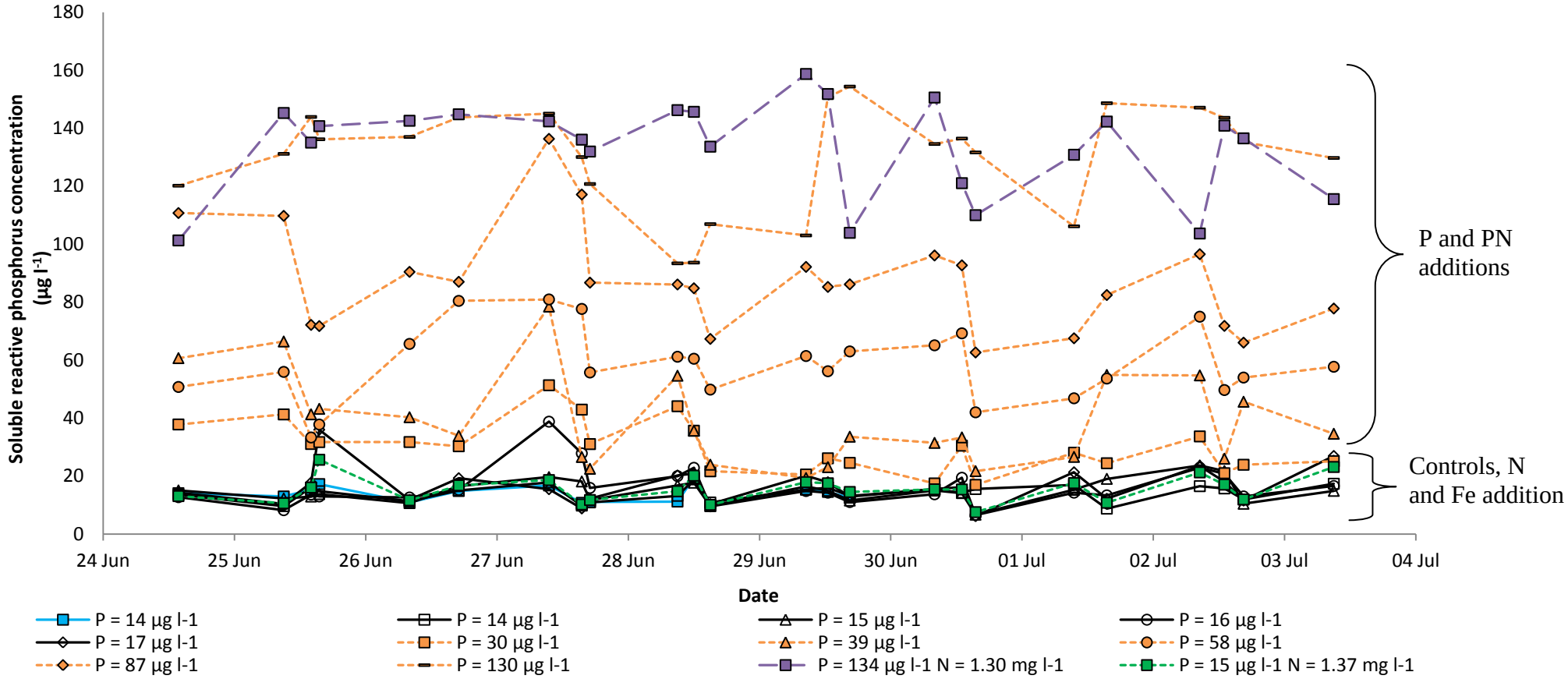


Figure 4.4: Soluble reactive phosphorus (SRP) concentration in each flume over the course of the nine day nutrient manipulation experiment.. Solid blue line with filled symbol = iron addition, solid black line with open symbol = control (no addition), dotted green line with filled symbol = nitrogen addition (no phosphorus), dotted orange line with filled symbol = phosphorus addition (no nitrogen) and dashed purple line with filled symbol = phosphorus and nitrogen addition.

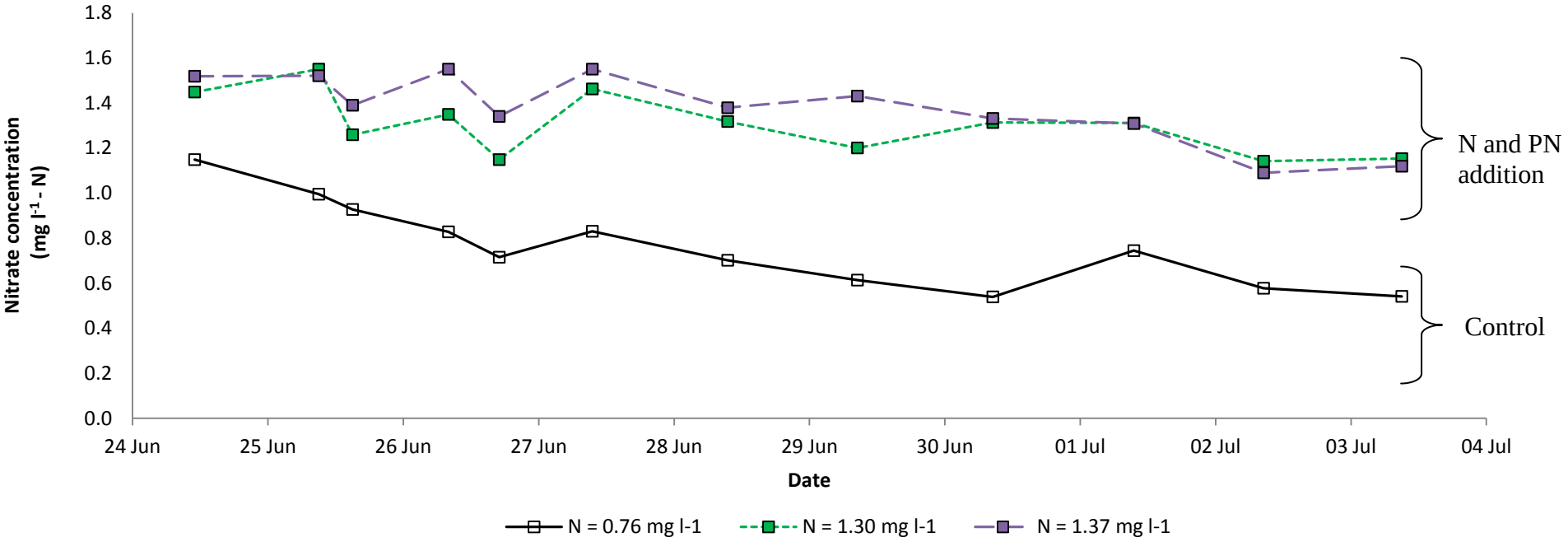


Figure 4.5: Nitrogen concentrations observed in the two flumes receiving nitrogen and one control flume (no addition). Solid black line with open symbol = control (no addition), dotted green line with filled symbol = nitrogen addition (no phosphorus) and dashed purple line with filled symbol = phosphorus and nitrogen addition.

4.3.3 Periphyton biomass response

The rate of periphyton accrual within each flume, estimated by chlorophyll-*a* concentration, is shown in Figure 4.6. There was little difference between treatments for the first three days of the experiment. All flumes continued to accrue periphyton biomass at a similar rate except for the flume receiving the combined phosphorus and nitrogen treatment, in which growth rate was approximately three times greater than the other nutrient treatments (Figure 4.6).

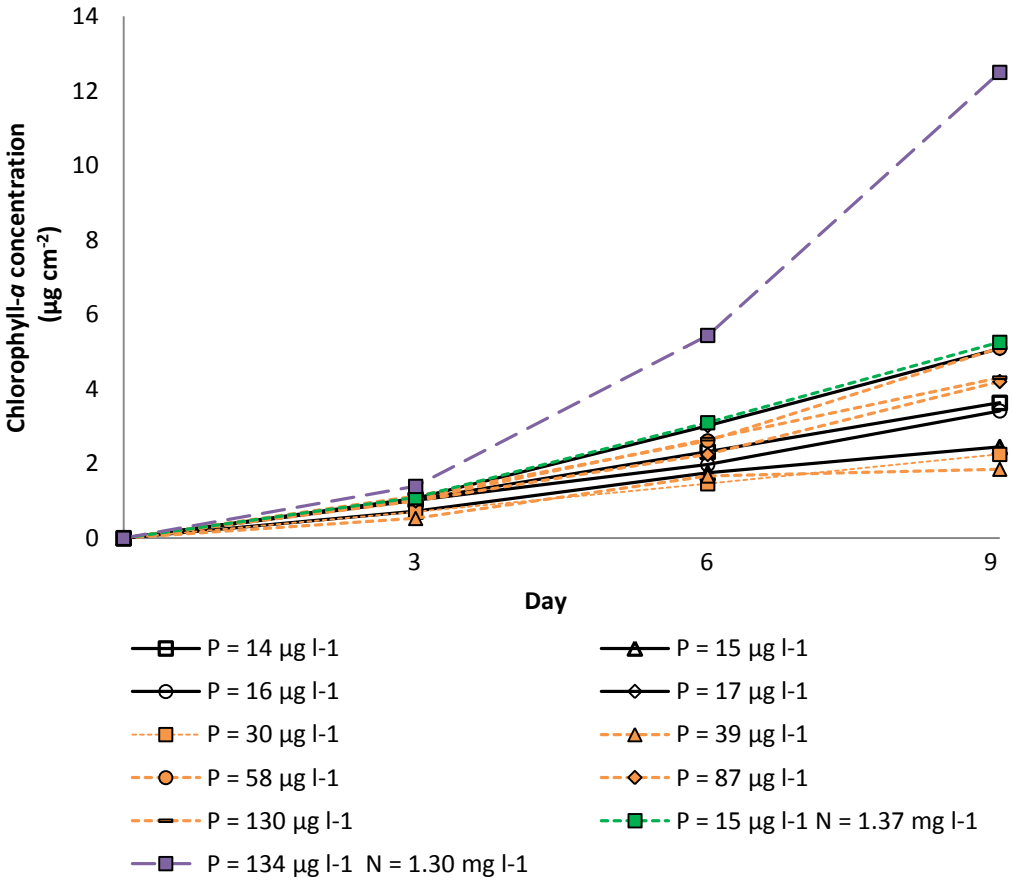


Figure 4.6: Rate of periphyton biomass accrual (as indicated by chlorophyll-*a* concentration) in individual flumes throughout the nine day experiment.

Total periphyton biomass, in terms of chlorophyll-*a* and AFDM in response to nutrient enrichment, is shown in Figure 4.7 and Figure 4.8. Statistical analysis (ranged major axis regression), of the quantities of periphyton accrued on the tiles on

day nine of the experiment showed that up to a nine-fold increase in river SRP concentration (from 15 to 130 µg l^{-1}) had no significant effect on chlorophyll-*a* concentration or AFDM (chlorophyll-*a*: statistic = 0.43, p = 0.09; AFDM: statistic = 0.55, p = 0.12). The mean chlorophyll-*a* concentrations in the control flumes were between 2.44 and 3.63 µg cm^{-2}. These increased slightly to 4.31 µg cm^{-2} when the SRP concentration was increased to 130 µg l^{-1}, but a doubling of SRP concentration (to 39 µg l^{-1}) actually reduced the chlorophyll-*a* concentration (Figure 4.7), demonstrating that the River Rede was not phosphorus limited. Similarly, the mean AFDM in the control flumes were between 0.85 and 1.44 mg cm^{-2}. AFDM increased to 1.56 mg cm^{-2} at a SRP concentration of 130 µg l^{-1}, but when normalised to the AFDM in the control flumes (to take into account variations between flume sets), this again showed that increased SRP concentrations did not increase periphyton accrual rate (Figure 4.8B).

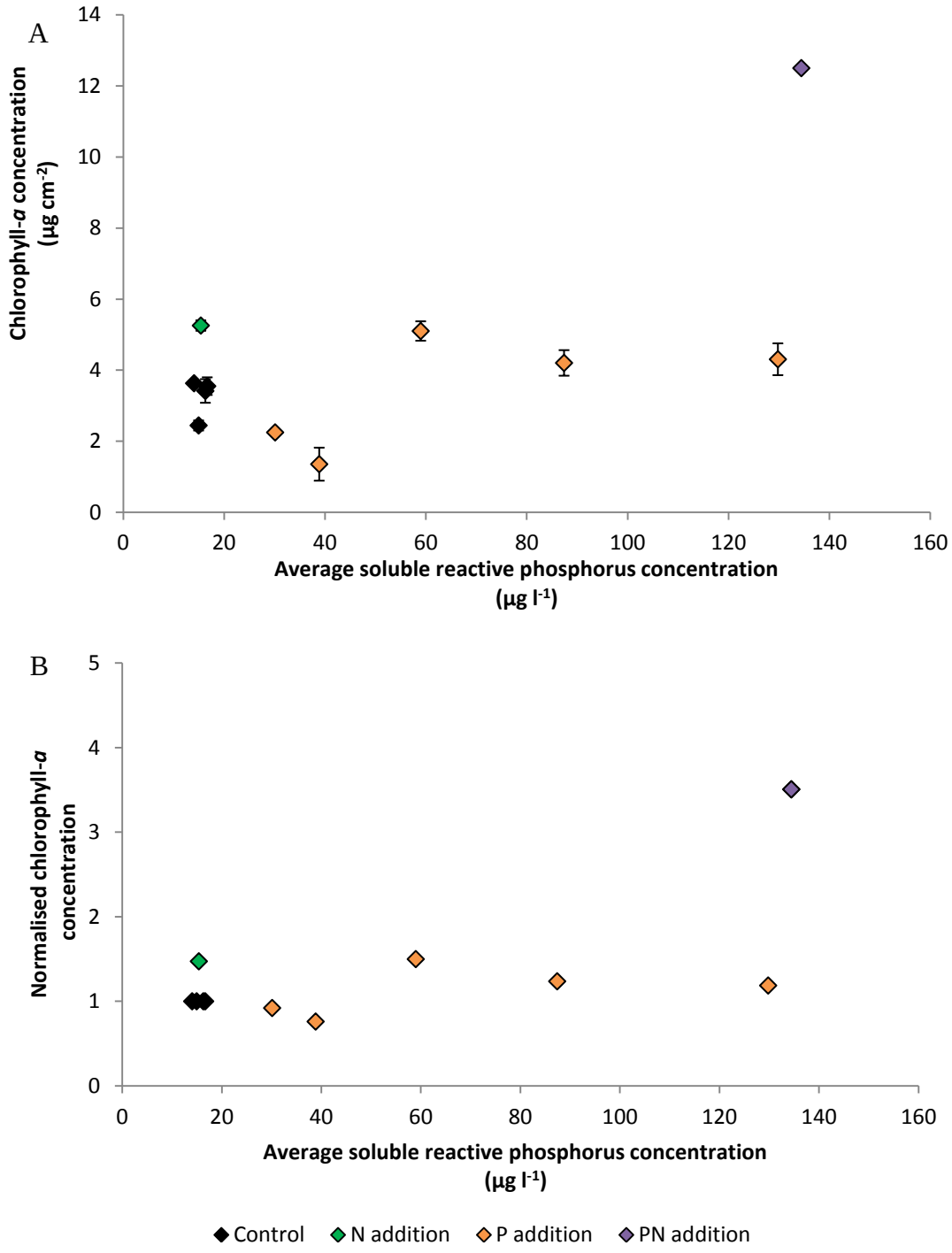


Figure 4.7: Relationship between soluble reactive phosphorus concentration and (A) chlorophyll-*a* concentration after nine days (data points are mean values based on analysis of three tiles ± one standard error) and (B) chlorophyll-*a* concentration normalised to the control in each set of three flumes.

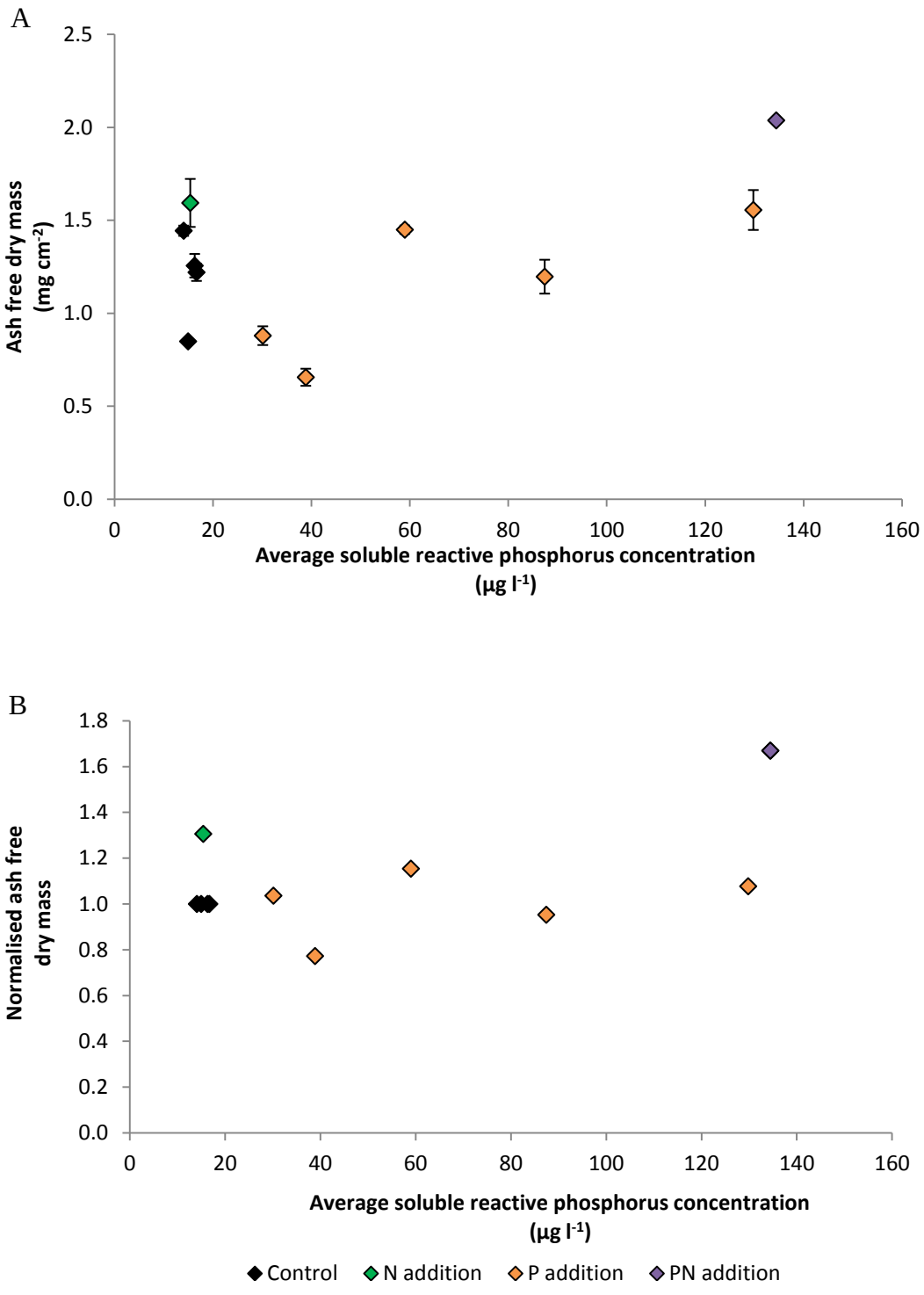


Figure 4.8: Relationship between soluble reactive phosphorus concentration and (A) ash free dry mass after nine days (data points are mean values based on analysis of three tiles ± one standard error) and (B) ash free dry mass normalised to the control in each set of three flumes.

Even in this nutrient poor system, the above data have demonstrated that phosphorus concentration was not limiting periphyton growth, as indicated by the lack of significant increase in chlorophyll-*a* concentration and AFDM with increasing SRP concentrations. Yet, water N: P ratios calculated in the control flumes and low phosphorus addition treatments (i.e. SRP concentration below 40 µg l^{-1}) (Table 4.2) suggest ambient summer phosphorus concentrations could be limiting to periphyton growth (based on the Redfield ratio of 16: 1) (Redfield, 1958). This suggests (as discussed in Section 1.9) that N: P ratios are not an effective means of predicting nutrient limitation (Keck and Lepori, 2012). However, chlorophyll-*a* concentrations from the experiment were much lower than those of previous experiments, suggesting that some other factor may have limited periphyton growth.

An 80 % increase in ambient nitrogen concentration gave a 48 and 30 % increase in chlorophyll-*a* concentration and AFDM to 5.26 µg cm^{-2} and 1.59 mg cm^{-2}, respectively (Figure 4.7 and Figure 4.8). This increase cannot be tested statistically (due to lack of a nitrogen gradient or appropriate replication), but indicates some degree of nitrogen limitation. Adding both phosphorus (134 µg l^{-1}) and nitrogen (1.30 mg l^{-1}) simultaneously, resulted in a 3.5-fold increase in chlorophyll-*a* concentration to 12.50 µg cm^{-2} (Figure 4.7), and a 62 % increase in AFDM to 2.04 mg cm^{-2} (Figure 4.8), suggesting nutrients are sequentially co-limiting periphyton biomass in the River Rede (Figure 1.5).

On the final day of the experiment, the autotrophic index (AI) values from the four control flumes were between 356 and 410. The addition of phosphorus did not affect this, with values ranging from 292 to 401. Adding phosphorus and nitrogen simultaneously, resulted in a much lower AI of 167 suggesting that this simultaneous phosphorus and nitrogen addition is increasing the proportion of chlorophyll-containing autotrophs within the biofilm (Ameziane *et al.*, 2002).

The River Rede experiment is the first time that flume-based nutrient addition experiments (previously applied to English rivers with SRP concentrations ranging from 45 µg l^{-1} to 230 µg l^{-1}) have shown a response in periphyton growth from any form of nutrient enrichment. As only one flume was exposed to each of the nitrogen and combined phosphorus / nitrogen treatments, further work would need to be undertaken to confirm the sequential co-limitation of the system (see Section 1.9.1).

However, the possible sequential co-limitation of periphyton biomass concurs with recent studies that indicate that occurrences of phosphorus and nitrogen co-limitation were significantly greater than limitation by phosphorus or nitrogen individually (Elser *et al.*, 2007, Harpole *et al.*, 2011). Co-limitation was also determined to be more common in environments where ambient concentrations of phosphorus and nitrogen were low (Harpole *et al.*, 2011), as in the case of the River Rede.

The sequential co-limitation observed was defined by Elser *et al.* (2009) as being a situation where periphyton biomass responds to a single nutrient addition (phosphorus or nitrogen) but exhibits a greater response to a combined nutrient addition (phosphorus and nitrogen) (see Section 1.9.1). In the River Rede, the addition of nitrogen increased chlorophyll-*a* by 48 % and AFDM by 13 %. Yet the periphyton biomass response as a result of adding the two nutrients in combination was much greater with *ca.* 250 % increase in chlorophyll-*a* concentration and a 140 % increase in AFDM. Therefore, it is plausible to say the specific sequential co-limitation observed is that of nitrogen. The biomass response in the control treatment (Chl_{Con}) is equal to that of the phosphorus treatment (Chl_{P}) and both treatments have less of a biomass response than the nitrogen addition treatment, which in turn is less than the biomass response when phosphorus and nitrogen were added simultaneously (Chl_{PN}) (Elser *et al.*, 2009) (Figure 4.9).

$$Chl_{Con} = Chl_{P} < Chl_{N} < Chl_{PN}$$

Figure 4.9: Schematic diagram representing the response of periphyton in terms of chlorophyll (Chl) concentration to different forms of nutrient enrichment in the River Rede.

4.3.4 Periphyton phosphorus concentration

Although there was no significant relationship between total periphyton biomass and SRP concentration in the overlying river water (Figure 4.7 and Figure 4.8), there was a significant linear relationship between mean river water SRP concentration and periphyton phosphorus concentration in each flume (statistic – 0.79, $p = 0.001$) (Figure 4.10). At ambient SRP concentrations, stored phosphorus concentration

within the periphyton biofilm was 2.90 µg mg $AFDM^{-1}$ (S.E. = 0.26). A nine-fold increase in SRP concentration from *ca.* 15 µg l^{-1} to a mean of 130 µg l^{-1} resulted in a three-fold increase in periphyton phosphorus concentration from 2.84 µg mg $AFDM^{-1}$ (S.E. = 0.06) to 8.65 µg mg $AFDM^{-1}$ (S.E. = 0.53). Similar results were reported by Lohman and Priscu (1992) who examined nutrient limitation across seasons in Montana and found internal (stored) nutrient concentrations followed the same pattern as nutrient concentrations in the water column.

The biofilm grown in the nitrogen addition treatment had a mean periphyton phosphorus concentration that was 15 % less than the mean of the control treatments (nitrogen treatment periphyton phosphorus concentration = 2.42 µg mg $AFDM^{-1}$, S.E. = 0.07; control treatment periphyton phosphorus concentration = 2.84 µg mg $AFDM^{-1}$, S.E. = 0.06 - Figure 4.10) despite having similar SRP concentrations for the duration of the experiment (Figure 4.4). The mean periphyton phosphorus concentration of the biofilm in the flume receiving combined phosphorus and nitrogen addition was also slightly lower than when phosphorus was added alone at a similar concentration (7.30 µg mg $AFDM^{-1}$, S.E. = 0.15 for the phosphorus and nitrogen addition flume, compared to 8.65 µg mg $AFDM^{-1}$, S.E. = 0.53 for the phosphorus addition flume - Figure 4.10). If the system is sequentially co-limited, these slightly lower values for the treatments receiving nitrogen additions can be explained when examined in conjunction with total biomass measurements, which were all higher in treatments involving nitrogen. In these nitrogen addition treatments, stored phosphorus was being utilised, along with nitrogen in the overlying water, to produce new biomass, and therefore the concentration of phosphorus per milligram biomass will be reduced.

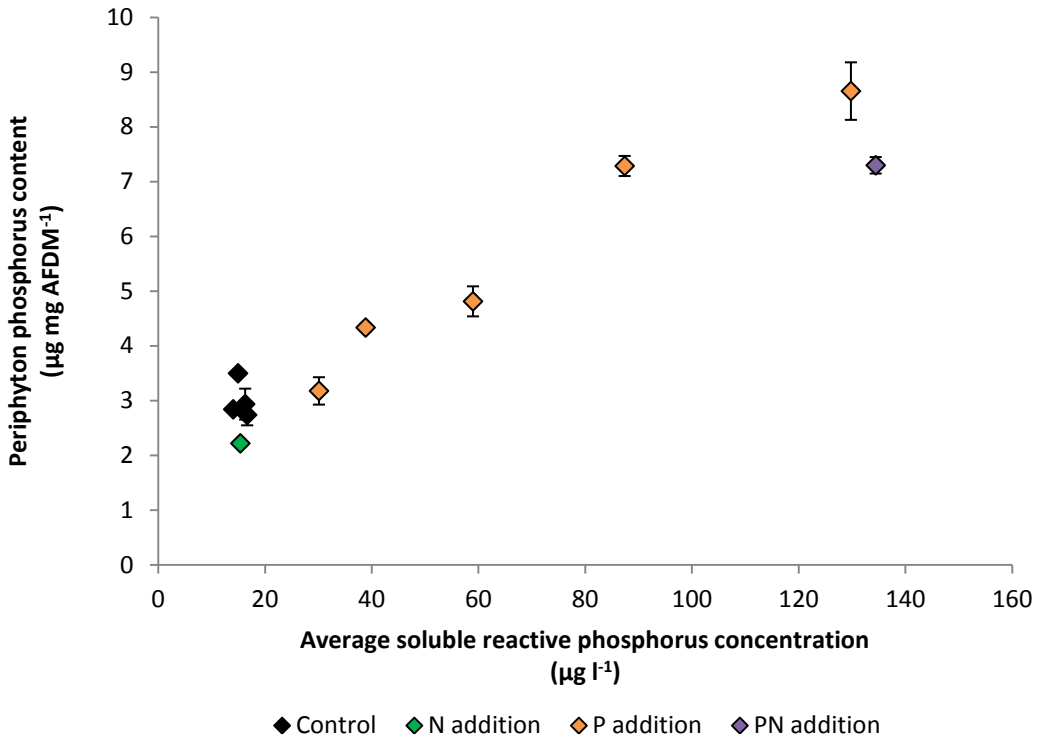


Figure 4.10: Periphyton phosphorus content for tile substrates after the nine day experiment across the entire range of nutrient concentrations. Data points are mean values based on analysis of three tiles from each flume ± one standard error.

Analysis of N: P ratio within periphyton cells from each nutrient treatment (Table 4.3) provides further evidence that excess phosphorus in the water column was being stored within the periphyton cells. If phosphorus was being used for growth (and increasing biomass accrual) it would be expected that the N: P ratio would be the same across all phosphorus treatments. However, as Table 4.3 shows, this was not the case. As water SRP concentration increased, the N: P ratio within the biofilm decreased proving that there is more phosphorus within the periphyton cells relative to nitrogen. The ratios shown in Table 4.3 are generally similar to those presented in Table 4.2 which shows water column N: P ratios. The exception to this was in the nitrogen addition treatment where water N: P ratio was 91 and periphyton N: P ratio was 41. The lower ratio in the periphyton sample indicates that less phosphorus is being stored than would be expected based on water column ratios. This agrees with findings presented in Figure 4.7, Figure 4.8 and Figure 4.10 whereby in the nitrogen addition treatment, periphyton biomass accrual was greater and stored phosphorus

concentration was lower. This is related to the sequential nutrient co-limitation of the system. Periphyton cells were utilising the added nitrogen along with sequestered phosphorus to produce new biomass.

Table 4.3: Periphyton N: P ratios on the final day of the nutrient manipulation flume experiment across all nutrient treatments. Numbers is brackets were inferred rather than measured.

Nutrient treatment	Average water nutrient concentration		Solid N: P ratio
	SRP ($\mu g\ l^{-1}$)	NO_3 - N ($mg\ l^{-1}$)	
P addition	58	(0.76)	15: 1
Control	16	(0.76)	32: 1
P addition	87	(0.76)	7: 1
Control	15	(0.76)	30: 1
P addition	39	(0.76)	18: 1
P addition	30	(0.76)	29:1
Control	14	(0.76)	32: 1
P addition	130	(0.76)	9: 1
P and N addition	134	1.30	13: 1
N addition	15	1.37	41: 1
Control	17	0.76	39: 1

These observations indicate that individual spikes in SRP concentration in the River Rede would not immediately result in a benthic algal bloom. However, if this excess phosphorus was being stored within the periphyton cells, subsequent spikes in nitrogen concentration may have the potential to increase periphyton growth rates. This important observation should be investigated in future experiments to determine how the periphyton responds to intermittent phosphorus and nitrogen spikes of different concentrations and durations.

4.3.5 Diatom assemblages

Regression analysis (ranged major axis regression) of the trophic diatom index against average SRP concentration showed a significant linear relationship between these two variables ($p = 0.02$, statistic = 0.14). The lowest TDI values (47 to 54)

were observed in the control treatments (ambient SRP *ca.* 15 µg l^{-1} (Figure 4.11)). At higher SRP concentrations (> 100 µg l^{-1}), the TDI increased to 60, indicating that at high SRP concentrations there was a growing proportion of more nutrient-tolerant diatom species (see Appendix D for names, abundance and sensitivity of all species identified and TDI calculations). Diatoms classed by the TDI as group 5 sensitivity (i.e. tolerant to high nutrient loads) comprised 23.8 % of the total count at higher SRP concentrations (130 µg l^{-1}) and dropped to between 9.6 and 14.6 % at all other nutrient concentrations. One species in particular, *Achnanthidium minutissimum*, which is known to be sensitive to pollutants, showed a marked decline in abundance with increasing SRP concentrations (from 16.0 to 6.5 % of the total count at SRP concentrations of 15 and 130 µg l^{-1} respectively).

A further indication of change in species composition was given by the percentage of motile species (Figure 4.11), whose abundance increased at all phosphorus concentrations above the ambient concentration. Increasing abundance of motile species with organic and inorganic phosphorus enrichment has been observed previously (Pringle, 1990, Lange *et al.*, 2011). Motile species have been described as "superior competitors for nutrients in nutrient-rich environments" (Van der Grinten *et al.*, 2004). Motility allows diatoms to exploit the increase in phosphorus concentration in the overlying water. It also allows a competitive advantage for light in thicker biofilm mats (which were observed at higher SRP concentrations) (Lange *et al.*, 2011). Motile diatoms are generally larger, allowing an increased phosphorus storage capacity (Pringle, 1990). As Section 4.3.4 shows, the periphyton communities in the River Rede were able to store more phosphorus at higher concentrations.

An average of 50 species per sample were identified. The most commonly identified species were *Nitzschia acicularis, Achnanthidium minutissimum, Fragilaria vaucheriae, Nitzschia palea* and *Encyonema minutum*. Assemblage differences were observed between the different treatments (see Appendix D) though the dominance of *Achnanthidium minutissimum* and *Fragilaria vaucheriae* throughout all samples is an indication of the overall good ecological status of the River Rede (Kelly *et al.*, 2008).

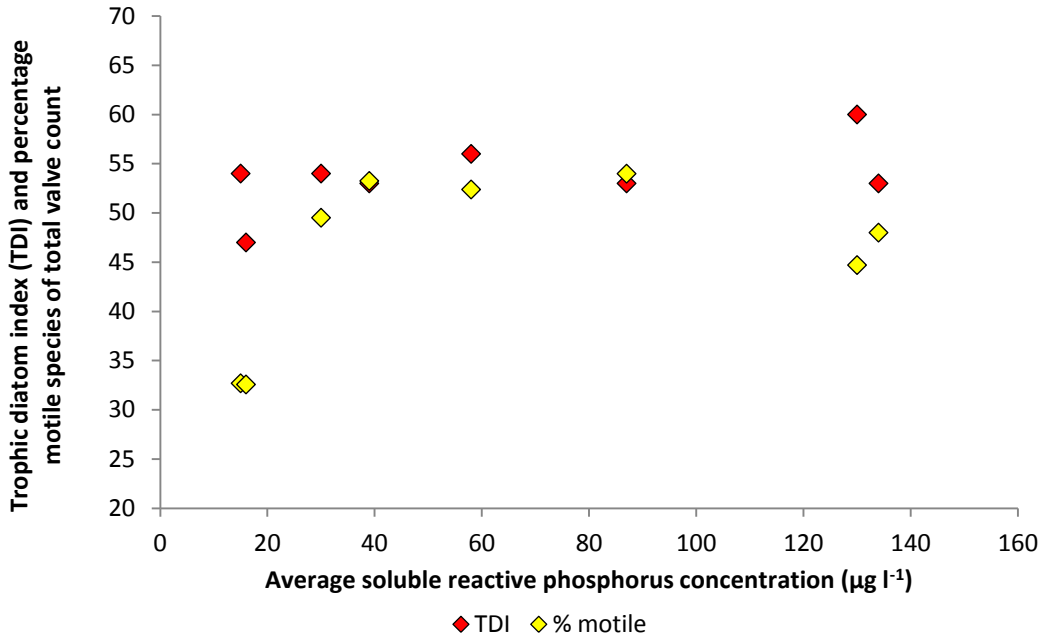


Figure 4.11: Trophic diatom index scores and the percentage motile diatoms present within the biofilm for each nutrient treatment.

4.3.6 Observed differences between the flumes and the river

The excessive periphyton growth that was observed in the control flumes towards the end of the experiment was not representative of that observed in the main river channel, despite the water chemistry being the same. There are three possible reasons for this. Firstly, periphyton biomass in the river could be regulated by top-down control due to the influence of grazers (Feminella and Hawkins, 1995, Hillebrand, 2002) (which were largely excluded from the flume mesocosms). Secondly, the periphyton on the river bed could be limited by light (Corkum, 1996b, Hill *et al.*, 2009, Hill *et al.*, 2011). There are extensive areas of peat within the upper Rede catchment, resulting in the water of the River Rede being highly coloured. To illustrate this, a light / depth profile of the River Rede during the flume experiment showed light intensity to be 20 937 Lx at a depth of 5 cm below the water's surface (the same water depth as the flumes), decreasing rapidly to 4 846 Lx at the river bed (a depth of 85 cm). The rapid attenuation of light levels with river depth could play a major role in limiting benthic algal growth within the river. Finally, the water velocity within the flume mesocosms was approximately half of the mean velocity measured in the main river channel during base flow conditions. Therefore, the

influence of scouring and effects of disturbance of periphyton biomass would be greatly reduced in the flume mesocosms.

4.4 River water quality

The water quality data from the longitudinal survey of the River Rede on 1st July 2011 are presented in Table 4.4 (site locations are shown in Figure 4.1). There was a general increase in nutrients (phosphorus and nitrogen) with distance downstream. TP concentration was 6 µg l^{-1} upstream of Catcleugh Reservoir (Site 1), increasing to 22 µg l^{-1} at West Woodburn (Site 7) and Redesmouth (Site 8).

A spike in SRP concentration of 30 µg l^{-1} was observed 100 m downstream of the STW at Otterburn (Site 5), due to effluent inputs (TP and SRP concentrations in the final effluent were 6270 µg l^{-1} and 4000 µg l^{-1} respectively). Such a spike would not be expected to impact significantly on river ecology, as river concentrations returned to 13 µg l^{-1} (750 m downstream) due to rapid sequestration by sediment and biota (nutrient spiralling) (Newbold *et al.*, 1983, Bowes and House, 2001, Jarvie *et al.*, 2012). Between Otterburn and the confluence with the North Tyne at Redesmouth, phosphorus concentrations remained relatively stable with SRP and TDP concentration being between below 15 µg l^{-1} and TP concentration being 22 µg l^{-1}.

The nitrate-N concentration in the River Rede increased from 0.2 mg l^{-1} upstream of Catcleugh Reservoir (Site 1) to 0.7 mg l^{-1} at West Woodburn (Site 7). Elsdon Burn (Site 6), which joins the River Rede between Otterburn and West Woodburn, had a slightly higher nitrogen concentration of 1.2 mg l^{-1}. However, due to the effects of nutrient spiralling, uptake by biota and dilution, the increased nitrogen concentration was not detected at the West Woodburn sampling site a further 9 km downstream. The boron concentration (an indicator of sewage input) of the river also increased downstream from 11.2 µg l^{-1} to 19.4 µg l^{-1}.

These results suggest that increases in nutrient concentration were low and insignificant when compared to the nutrient treatments produced in the flume experiment (Table 4.2), suggesting that nutrient concentrations are likely to sequentially co-limit periphyton growth rate along the entire length of the River Rede.

Table 4.4: Water chemistry data from the longitudinal survey conducted on 1st July 2011.

Site	Site location	River	Distance downstream (km)	Soluble reactive P (µg l^{-1})	Total dissolved P (µg l^{-1})	Total P (µg l^{-1})	Ammonium (mg l^{-1})	Nitrate - N (mg l^{-1})	Dissolved reactive silicon (mg l^{-1})	Chloride (mg l^{-1})	DOC (mg l^{-1})	pH	Alkalinity (µequiv l^{-1})	Sodium (mg l^{-1})	Potassium (mg l^{-1})	Calcium (mg l^{-1})	Magnesium (mg l^{-1})	Boron (µg l^{-1})	Iron (µg l^{-1})	Manganese (µg l^{-1})	Zinc (µg l^{-1})	Copper (µg l^{-1})	Aluminium (µg l^{-1})
1	Upstream of Catcleugh	Rede	2.6	11	6	6	0.017	0.2	1.8	6.9	2.7	7.83	1859	4.8	0.9	25.4	9.1	11.2	225	16.5	2.8	1.4	31.9
2	Rochester	Sills Burn	18.7	19	20	40	0.066	0.1	1.1	6.8	8.0	7.79	2318	5.0	1.5	35.1	10.8	24.1	1094	38.8	4.3	3.5	37.4
3	Elishaw Bridge	Rede	24.2	< LD*	9	25	< LD*	0.1	0.9	8.2	7.0	7.52	1767	6.1	1.1	27.7	8.5	19.4	517	43.4	9.0	1.4	33.8
4	Otterburn	Rede	29.6	13	5	10	0.039	0.4	1.1	10.4	7.5	7.65	1745	7.0	1.2	28.5	7.4	17.5	640	83.9	3.2	2.0	47.8
5	Downstream of Otterburn	Rede	29.8	30	39	68	0.069	0.7	1.1	11.1	7.8	7.49	1759	7.5	1.2	28.3	7.3	17.6	640	87.2	3.7	2.1	43.8
6	Monkridge Farm	Elsdon Burn	32.4	7	8	22	0.014	1.2	1.1	12.9	4.7	7.86	2918	8.8	1.7	58.9	8.9	22.4	504	80.6	0.3	1.5	6.7
7	West Woodburn	Rede	39.1	6	15	22	0.019	0.7	2	11	7.8	7.89	1803	7.6	1.2	30.9	6.7	18.6	782	35.4	1.2	1.5	40.0
8	Redesmouth	Rede	57.8	15	13	22	0.013	0.5	1.2	11.2	8.6	7.93	1794	7.9	1.3	30.9	6.6	19.4	737	37.7	2.3	1.4	42.0
	Otterburn STW	Waste stream	29.7	4000	6270	6840	39	13.6	4.1	79.9	51.4	N/A	N/A	79.6	18.1	37.8	6.9	57.9	185	131.3	39.4	< LD*	< LD*

* < Limit of detection

4.5 Conclusions

The present study clearly demonstrates that, even in a river with some of the lowest phosphorus concentrations in England, a sustained nine-fold increase in SRP concentration had no effect on total periphyton biomass or rate of accrual, and that phosphorus concentration was, therefore, not the primary factor limiting total periphyton biomass. The present experiment did, however, show that an increase in SRP concentration led to a change in the diatom community, with a significant increase in the TDI (one of the indices being used to measure progress against the WFD). This finding agrees with that of Chapter Three on the River Lambourn in that the diatom community within the periphyton biofilm is affected by nutrient concentrations, even when periphyton accrual was not, and provides further evidence for a lower ecological phosphorus threshold.

Similar experiments on English rivers with widely varying levels of nutrient enrichment have all shown that an increase in phosphorus concentration has never resulted in a corresponding increase in periphyton biomass and accrual rate. This poses serious questions for the current national and international mitigation strategies that are very much focussed on phosphorus reduction. It also suggests that current SRP concentrations are unlikely to have been the cause of the failure in recruitment of the River Rede's freshwater pearl mussel population over the last 30 years. There is clearly a need to consider other abiotic variables known to affect periphyton growth, including flow regime, light intensity, food-web interactions and sedimentation.

The present work suggests the need for future management of the River Rede catchment to take a balanced approach to the abatement of both phosphorus and nitrogen. As this study has shown, phosphorus did not limit total periphyton biomass in the river, but elevated concentrations of both phosphorus and nitrogen could result in an increase in periphyton biomass accrual. It may be particularly important to control nitrogen concentrations downstream of STW, as the peaks in phosphorus caused by waste-effluent discharge into the river and the ability of periphyton to store excess phosphorus make this part of the river ecosystem particularly vulnerable to increased periphyton growth.

Chapter 5: Do periphyton biofilm communities adapt to their nutrient environment?

5.1 Introduction

The River Frome, Dorset, is one of the few rivers in the UK with weekly water chemistry data stretching back over 40 years (Bowes *et al.*, 2011a, Bowes *et al.*, 2011b), produced by the Freshwater Biological Association, Institute of Freshwater Ecology and Centre for Ecology and Hydrology. The dataset means that long-term and step-changes in nutrient concentrations have been captured in time, providing a wealth of background data to support interpretation of shorter-term studies. Specifically, the dataset has made it possible to link water quality changes with changes in land use and STW improvements in the catchment. As a result, the catchment has been intensively studied, covering a wide range of subjects including water quality (Casey and Newton, 1973, Casey and Clarke, 1986, Bowes *et al.*, 2005, Howden and Burt, 2008, Bowes *et al.*, 2009a, Howden *et al.*, 2010b, Bowes *et al.*, 2011a), high resolution nutrient monitoring (Bowes *et al.*, 2009b), method development (Casey *et al.*, 1989), fine sediment (Collins and Walling, 2007, Ballantine *et al.*, 2009), fish populations (Mann, 1989, Clough *et al.*, 1998, Welton *et al.*, 1999), invertebrates (Ladle *et al.*, 1977, Gunn, 1985, Dawson *et al.*, 1991) and how the biological community affects the physical environment (Clarke *et al.*, 2006, Cotton *et al.*, 2006, Gurnell *et al.*, 2006).

5.1.1 Changes in River Frome water quality (1965 – 2009)

The dataset (Bowes *et al.*, 2011b) documents how water quality in the River Frome has changed over a period of 44 years (1965 – 2009) at East Stoke (Site 14 on Figure 5.1). Nitrate was the most abundant form of nitrogen within the catchment with concentrations steadily increasing from a mean of 2.4 mg l^{-1} between 1965 to 1969 to a mean of 5.9 mg l^{-1} between 2000 and 2009 (Bowes *et al.*, 2011a). An increase in the use of industrial fertilisers, agricultural intensification (changes in ploughing practice) and an increase in atmospheric deposition have been cited as causes for this (Vitousek *et al.*, 1997, Hooda *et al.*, 2000, Howden and Burt, 2008). One of the main

effects of these factors has been contamination of chalk sub-aquifers leading to increased nitrogen concentrations in groundwater in the Frome catchment (Smith *et al.*, 2010). The rate of nitrogen concentration increase has slowed in recent years from 0.107 mg l^{-1} y^{-1} (1965 – 1975) (Casey and Clarke, 1979) to a rate of 0.83 mg l^{-1} y^{-1} (1976 to 2009) (Bowes *et al.*, 2011a). Furthermore, concentrations are unlikely to limit river ecology as concentrations over the 44 year dataset have always been in excess for periphyton growth (Redfield, 1958, Bowes *et al.*, 2011b).

Phosphorus concentrations have varied greatly over the monitoring period, initially increasing from a mean of 101 µg l^{-1} between 1965 and 1969 to a mean of 137 µg l^{-1} between 1970 and 1979. This was attributed to an increase in human population within the catchment (Bowes *et al.*, 2009a, Bowes *et al.*, 2011a). A peak in annual mean SRP concentration (190 µg l^{-1}) was observed in 1989, but since then mean concentrations have generally declined. The mean concentration between 2000 and 2009 was 86 µg l^{-1} (Bowes *et al.*, 2011a). The step-reductions in SRP concentration in the last decade have been attributed to the introduction of phosphorus-stripping at the major STW in the catchment (Dorchester in 2002 and Wool in 2006, – Site D and F respectively on Figure 5.1 (Bowes *et al.*, 2009a).

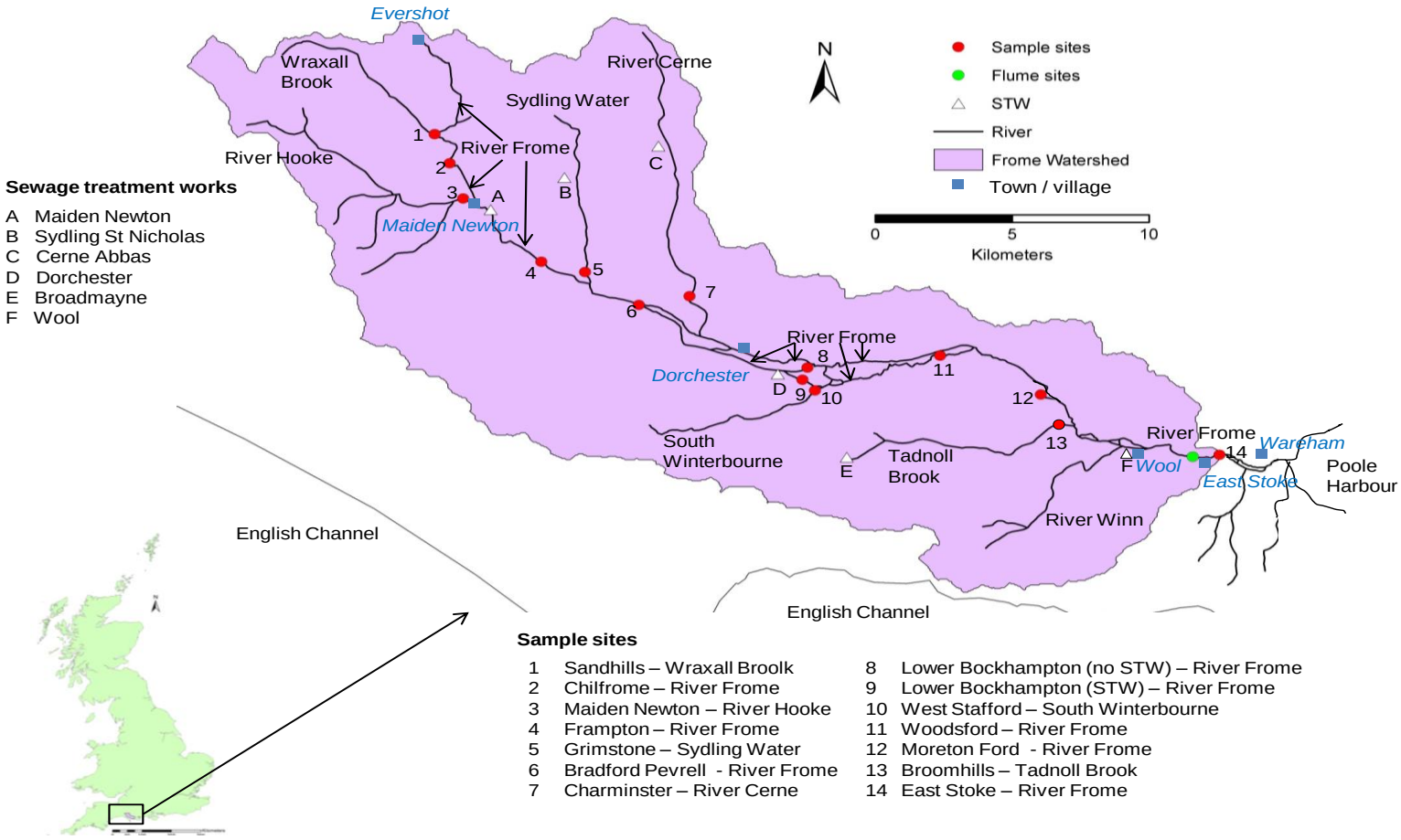


Figure 5.1: Map of River Frome catchment showing main tributaries, towns, sewage treatment works, sampling sites, and flume site. Insert shows location of Frome catchment within the United Kingdom. Numbers denote river sampling sites as part of a longitudinal survey. Letters denote sewage treatment works.

5.1.2 Current ecological status

As a result of continuing improvements in water quality, the River Frome is now classified as 'good' (in terms of its chemical quality) by the Environment Agency (Punchard, 2012). The river meets the UKTAG (2008) phosphorus target for lowland, high alkalinity (chalk) rivers of an annual average SRP concentration below 120 µg l^{-1} (UKTAG, 2008). However, the overall ecological quality of the River Frome is still classified as being 'poor' due to its fish communities which are likely to be influenced by high sediment loads (Punchard, 2012). Meeting the requirements of the WFD takes an 'all or nothing' approach with overall classification being based on the lowest individual standard (UKTAG, 2013b). A number of studies have found that improvements in water quality were not always coupled with expected ecological improvements (Kelly and Wilson, 2004, Neal *et al.*, 2010).

During the summer of 2005, two flume experiments (using streamside flumes) were conducted on the River Frome at East Stoke (see Figure 5.1 for location, Site 14 and Figure 5.2 for photograph) by Bowes *et al.* (2007), using similar methodology to that used in this thesis. The ambient SRP concentration (mean SRP between June and September 2005 – 120 µg l^{-1}) was both increased (to 420 µg l^{-1}) and decreased (to 32 µg l^{-1}, using iron dosing). Increases in SRP concentration had no effect on periphyton accrual rate, but decreases in SRP resulted in reduced biomass. This study concluded that the phosphorus-limiting threshold in the River Frome was a SRP concentration of *ca.* 90 µg l^{-1}. Ambient SRP concentrations must be reduced below this threshold before an observable ecological improvement would be seen (Bowes *et al.*, 2007).

Figure 5.2: Photograph of the 12 in-stream flume mesocosms used in the present experiment and the streamside flumes used in the study of Bowes *et al.* (2007).

5.1.3 Experimental aims and hypotheses

The flume methodology has since been applied to a heavily enriched river (River Thames), two moderately enriched rivers (River Kennet and River Lambourn) and one minimally enriched river (River Rede) using the portable flume mesocosms described in Section 2.1. The phosphorus-limiting threshold for periphyton biomass accrual in all of these rivers was below 100 µg l^{-1}. The Rivers Lambourn, Kennet and Frome had different ambient SRP concentrations of 45, 60 and 100 µg l^{-1} respectively, but all three rivers were at the phosphorus-limiting threshold. This could be a coincidence, but an alternative explanation could be that the periphyton communities in these moderately enriched rivers have adapted to produce maximum biomass at the current ambient SRP concentration. One way to test this would be to carry out multiple flume experiments in a number of different rivers with ambient SRP concentrations ranging from 40 to 100 µg l^{-1} to determine if they are all at the phosphorus-limiting threshold. Alternatively, if the phosphorus concentration at a site has been reduced since carrying out one of the flume experiments, it would be possible to undertake a further flume experiment to determine if the phosphorus concentration was now truly limiting or whether maximum biomass now accrues at

the new (lower) ambient phosphorus concentration (i.e. the phosphorus-limiting threshold changes to the new ambient SRP concentration) (Figure 5.3).

As a result of phosphorus-stripping being introduced at Wool STW on the River Frome (Site F - Figure 5.1) in 2006, SRP concentrations at the East Stoke experimental site, have reduced from 100 - 120 µg l^{-1} since the experiment of Bowes *et al.* (2007). In 2008, mean annual SRP concentration was 50 µg l^{-1} (Bowes *et al.*, 2011a). Between June and August 2011, the mean SRP concentration in the river was 68 µg l^{-1} at East Stoke, Site 14 on Figure 5.1 (data kindly provided by Wessex Water). The reduction in ambient SRP concentration in the River Frome provides an ideal opportunity to test the hypothesis that periphyton communities adapt to nutrient environments to accrue maximum biomass at ambient SRP concentrations (Figure 5.3).

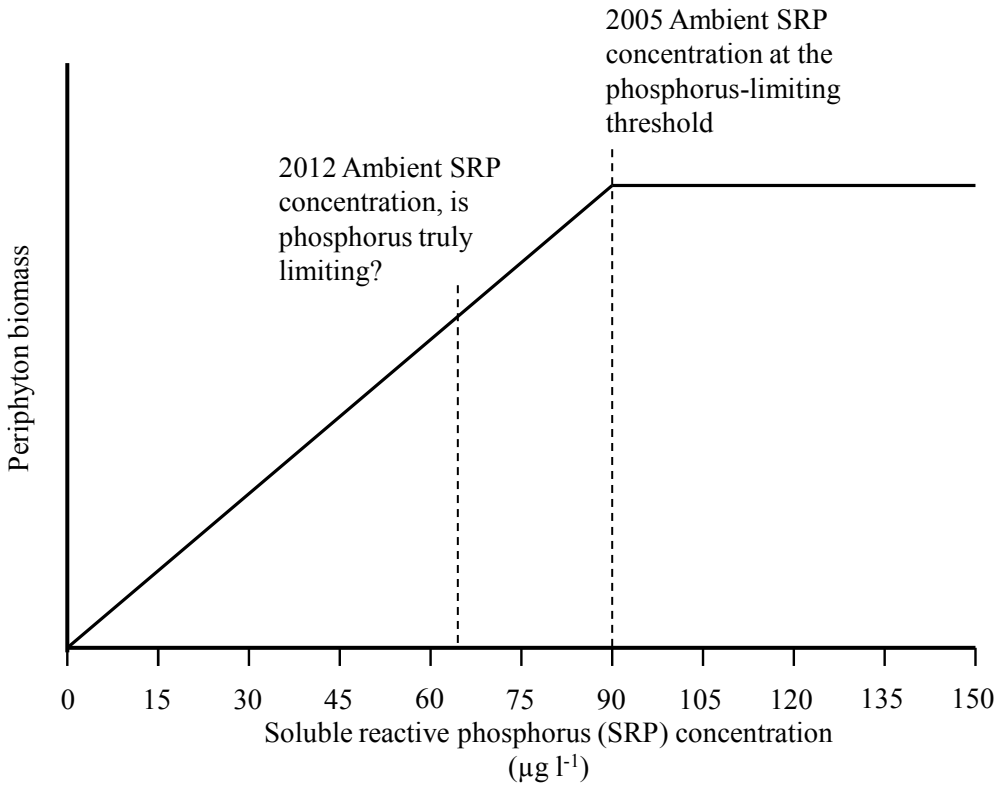


Figure 5.3: Schematic diagram representing the phosphorus-limiting threshold of the River Frome as identified by Bowes *et al.* (2007) and the predicted periphyton biomass response as a result of reduced ambient soluble reactive phosphorus concentrations in the river.

Based on the conclusions of Bowes *et al.* (2007), periphyton growth in the River Frome would now be expected to be truly phosphorus limited (Figure 5.3). Twelve portable in-stream flume mesocosms (described in Section 2.1) were installed in the Mill Stream (a branch of the River Frome), at the same location as the experiment of Bowes *et al.* (2007) in July 2012 (Figure 5.2). These were used to simultaneously increase and decrease nutrient concentrations to determine whether or not periphyton communities could adapt to reduced phosphorus concentrations. The specific hypotheses (and null hypotheses) tested (at a significance level of 0.05) were:

1. **H_1** – Increasing nutrient concentrations will lead to a significant increase in periphyton accrual and a more autotrophic community in the River Frome as indicated by an increase in chlorophyll-*a* concentration and ash free dry mass (AFDM) value and a decrease in autotrophic index (AI). There will also be an increase in the trophic diatom index (TDI).

   H_0 – Nutrients are not limiting periphyton growth in the River Frome. Therefore, increasing nutrients will have no significant effect on chlorophyll-*a* concentration, AFDM, AI or the TDI.
2. **H_1** – Decreasing nutrient concentrations will lead to a significant decrease in periphyton accrual and a less autotrophic community in the River Frome as indicated by a decrease in chlorophyll-*a* concentration and AFDM and an increase in AI. There will also be a decrease in the TDI

   H_0 – Decreasing nutrients will have no significant effect on chlorophyll-*a* concentration, AFDM, AI or the TDI.

5.1.4 Catchment description and study site

The source of the River Frome, Dorset is located at the village of Evershot and the river flows 59 km in a south easterly direction to its mouth at Poole Harbour (Figure 5.1) (Arnott *et al.*, 2009). The majority of the 414.4 km^2 catchment (Marsh and Hannaford, 2008) is underlain by Cretaceous Chalk bedrock with some Cretaceous Greensand in the River Hooke catchment and upper reaches of the Frome (Arnott *et al.*, 2009). In addition to the 44 year water quality dataset (Bowes *et al.*, 2011b), the River Frome catchment (similar to the River Lambourn) was intensively studied as part of the lowland catchment research (LOCAR) programme (Wheater *et al.*, 2007).

Between Dorchester and Wareham (49.04 km) (Figure 5.1), the Frome is designated as a Site of Special Scientific Interest due it being the most westerly chalk stream in Britain and due to the diverse, rare aquatic plant species including blunt-fruited water starwort, *Callitriche obtusangua*, spiked water milfoil, *Myriophyllum spicatum*, and blue water speedwell, *Veronica anagallis aquatica* (Punchard, 2012).

The Frome has a number of tributaries including Wraxall Brook, the River Hooke, the River Cerne, Sydling Water, South Winterbourne, Tadnoll Brook and the River Win. In addition, the main stem of the Frome is often split into different braided channels (Figure 5.1). Mean annual rainfall at East Stoke (Site 14) between 1965 and 2005 was 1020 mm with a resulting mean annual discharge of 6.83 m^3 s^{-1}. The base flow index of the river was 0.84, indicating the dominance of groundwater within the river system (Marsh and Hannaford, 2008). Ten STW discharge treated final effluent into the Frome (largest seven shown in Figure 5.1). The largest of these, Dorchester (P.E. of 27 600) and Wool (P.E. 8 000), had phosphorus-stripping introduced in 2002 and 2006, respectively. Agriculture is the main land use within the catchment (approximately 75 %) with more pasture (improved and semi-improved grassland) than arable. Woodland accounts for 10 % of catchment land use and unimproved grass land, shrub land and heath just over 5 %. The catchment is predominantly rural with urban areas accounting for less than 5 % of total land use. The total catchment population is approximately 50 000 with just over half (27 000) living in and around Dorchester and a further 9 000 people living in Wareham (Figure 5.1) (Punchard, 2012).

5.2 Experiment-specific methodology

Experiment 1 ran for seven days from 19th to 26th July 2012. Twelve flumes (four sets of three) were arranged in two sets of side by side pairs as shown in Figure 5.4. Flumes were secured in place using rope tied to scaffolding poles that were driven into the river bank.

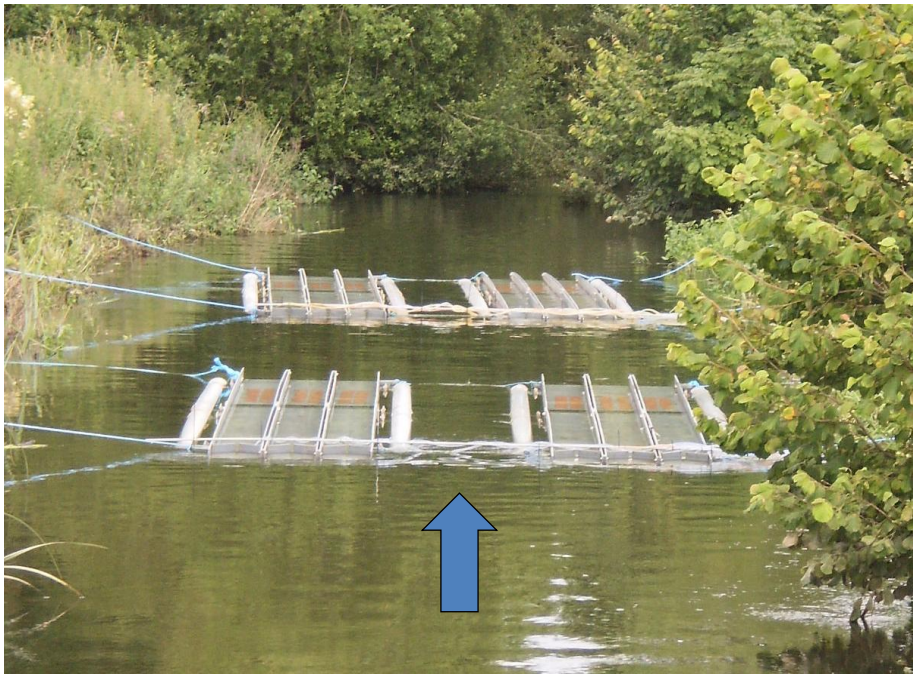


Figure 5.4: Arrangement of the twelve in-stream flume mesocosms in the experiment in the River Frome. The blue arrow represents direction of river flow.

Nutrient treatments were randomly assigned to each flume with one control being maintained in each set of three. All methods used were the same as the generic methods described in Sections 2.1 and 2.2. HOBO loggers were randomly placed in two flumes (one in each set of six) for the duration of the seven day experiment to record temperature and light intensity at hourly intervals. In addition, one logger was placed in the river at a similar depth to the flumes to record stream water temperature, one logger was attached to a tree to record air temperature and one logger was attached located in an unshaded position on the river bank adjacent to the flumes, to record light intensity and daylight hours. The flow velocity gates were set so the water velocity within each flume at the start of each experiment was 0.12 m s^{-1}. The target nutrient concentration in each flume in Experiment 1 is given in Table 5.1.

Table 5.1: Target nutrient concentrations during Experiment 1 on the River Frome from 19th to 26th July 2012. Increases and decreases were based on an ambient SRP concentration of 65 µg l^{-1} and NO_3-N concentration of 4.99 mg l^{-1}.

Flume number	Nutrient treatment	Target increase or decrease in SRP concentration (%)	Target increase in NO_3-N concentration (%)
1	PN addition	100	20
2	$FeCl_3$ addition	- 30	N/A
3	None (control)	N/A	N/A
4	P addition	30	N/A
5	None (control)	N/A	N/A
6	P addition	60	N/A
7	None (control)	N/A	N/A
8	$FeCl_3$ addition	- 60	N/A
9	P addition	150	N/A
10	N addition	N/A	20
11	None (control)	N/A	N/A
12	$FeCl_3$ addition	- 50	N/A

In order to achieve replication, the experiment was repeated (also seven day duration) between 27h July and 3rd August 2012 (Experiment 2). For this, the same nutrient concentrations were aimed for but the location of each treatment (within the flumes) was randomly assigned to a different place (Table 5.2).

Table 5.2: Target nutrient concentrations during Experiment 2 on the River Frome from 27th July to 3rd August 2012. Increases and decreases were based on an ambient SRP concentration of 65 µg l^{-1} and NO_3-N concentration of 4.99 mg l^{-1}.

Flume number	Nutrient treatment	Target increase or decrease in SRP concentration (%)	Target increase in NO_3-N concentration (%)
1	None (control)	N/A	N/A
2	PN addition	100	20
3	$FeCl_3$ addition	- 30	N/A
4	P addition	60	N/A
5	P addition	30	N/A
6	None (control)	N/A	N/A
7	$FeCl_3$ addition	- 60	N/A
8	P addition	150	N/A
9	None (control)	N/A	N/A
10	None (control)	N/A	N/A
11	$FeCl_3$ addition	- 50	N/A
12	N addition	N/A	20

5.3 Experiment 1 results and discussion

5.3.1 Light intensity and temperature

The light intensity and temperature measured in the flumes and the river (temperature only) is shown in Figure 5.5. For the duration of the experiment, tile substrates received light for 15 hours per day (6:00 am to 9:00 pm). Maximum light intensities ranged between 66 134 and 209 424 Lx (Figure 5.5A) with an average of 164 874 Lx throughout the course of the seven day experiment. The average light intensity during daylight hours was 68 859 Lx. Average temperatures in the two flumes in which it was measured were 16.46 ± 3.20 and 16.48 ± 3.42 °C. Mean river temperature for the duration of the experiment was 16.12 ± 3.08 °C. The temperatures in the flumes and the river were identical at night, but due to less water volume in the flumes and high air temperatures at the time of the experiment (up to 32.3 °C), flume temperatures were slightly higher during daylight hours (Figure 5.5B). However, a difference of less than 1 °C was unlikely to have affected periphyton biomass accrual and community structure.

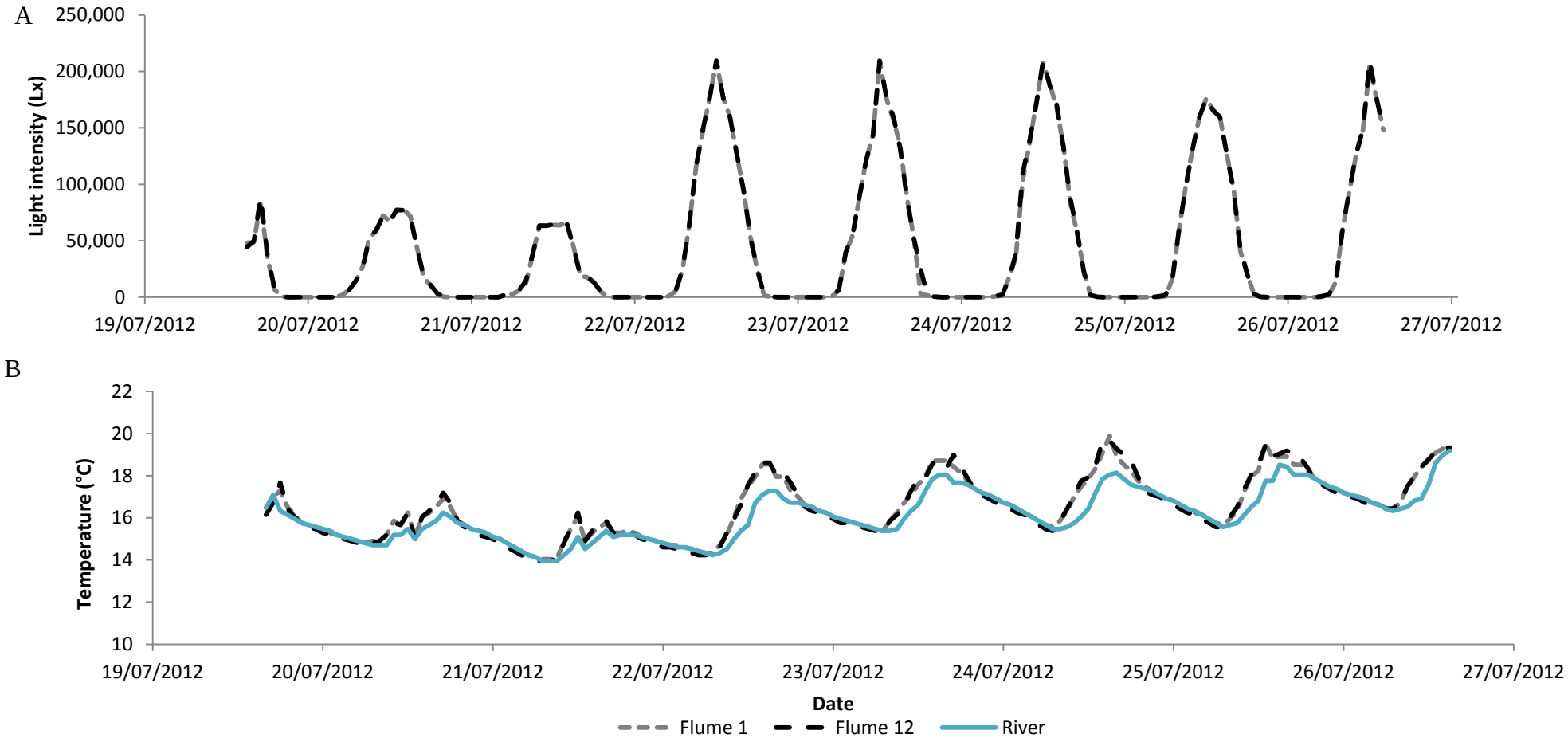


Figure 5.5: (A) Light intensity measured in two of the flumes at hourly intervals during the experiment. (B) Water temperature measured in two of the flumes and the River Frome at hourly intervals during Experiment 1.

5.3.2 Flume water chemistry

The nutrient concentrations measured in each flume for the duration of Experiment 1 are shown in Figure 5.6. The actual average concentrations achieved were within 12 % for phosphorus addition, 5 % for phosphorus reduction and 6 % for nitrogen addition of the target concentrations (Table 5.1). The mean SRP concentration in the four control flumes was 61.8, 62.8, 64.8 and 67.9 µg l^{-1}. The mean SRP concentration observed in the River Frome for the duration of the experiment was 72.9 µg l^{-1} (data recorded at 10 minute intervals; provided by Wessex Water) with a range in concentrations from 55.7 to 161.3 µg l^{-1}. Similar to SRP concentrations in the River Frome (Figure 5.7), SRP concentrations in the flumes remained around 60 µg l^{-1} for the majority of the experiment. However, there was a peak in SRP concentration beginning at 3:00 pm on 25th July 2012 and peaking at 10:00 pm the same day. SRP concentration remained high (*ca.* 90 µg l^{-1}) for the rest of the experiment (Figure 5.7) which was terminated at 3:30 pm on 26th July 2012. This sudden increase in SRP concentration was captured across all nutrient treatments in the flume experiment (Figure 5.6). This coincided with a nearby music festival, and probably reflects an overwhelming of the receiving STW from the influx of extra people to the catchment.

Phosphorus was added to three flumes increasing mean SRP concentrations over the seven day experiment to 83.7, 106.2 and 154.6 µg l^{-1}. The higher of these concentrations is similar to ambient concentrations regularly measured during the experiment of Bowes *et al.* (2007) while the lower concentration should be limiting to periphyton growth, based on the conclusions of the same experiment. Iron dosing reduced SRP concentration in three flumes by 25, 52 and 60 % to mean concentrations of 46.2, 32.6 and 25.9 µg l^{-1}.

Mean nitrate-N concentration measured in the river during the experiment was 4.99 mg l^{-1}. Two flumes received nitrogen additions for the duration of the experiment (one solely nitrogen and the other a combined nitrogen and phosphorus addition). This increased nitrogen concentration by 25 % to a mean of 6.24 mg l^{-1} in the flume just receiving nitrogen and by 14 % to a mean of 5.69 mg l^{-1} in the flume receiving nitrogen and phosphorus. The same flume had its SRP concentration increased by 110 % to a mean of 129.8 µg l^{-1} over the course of the seven day experiment.

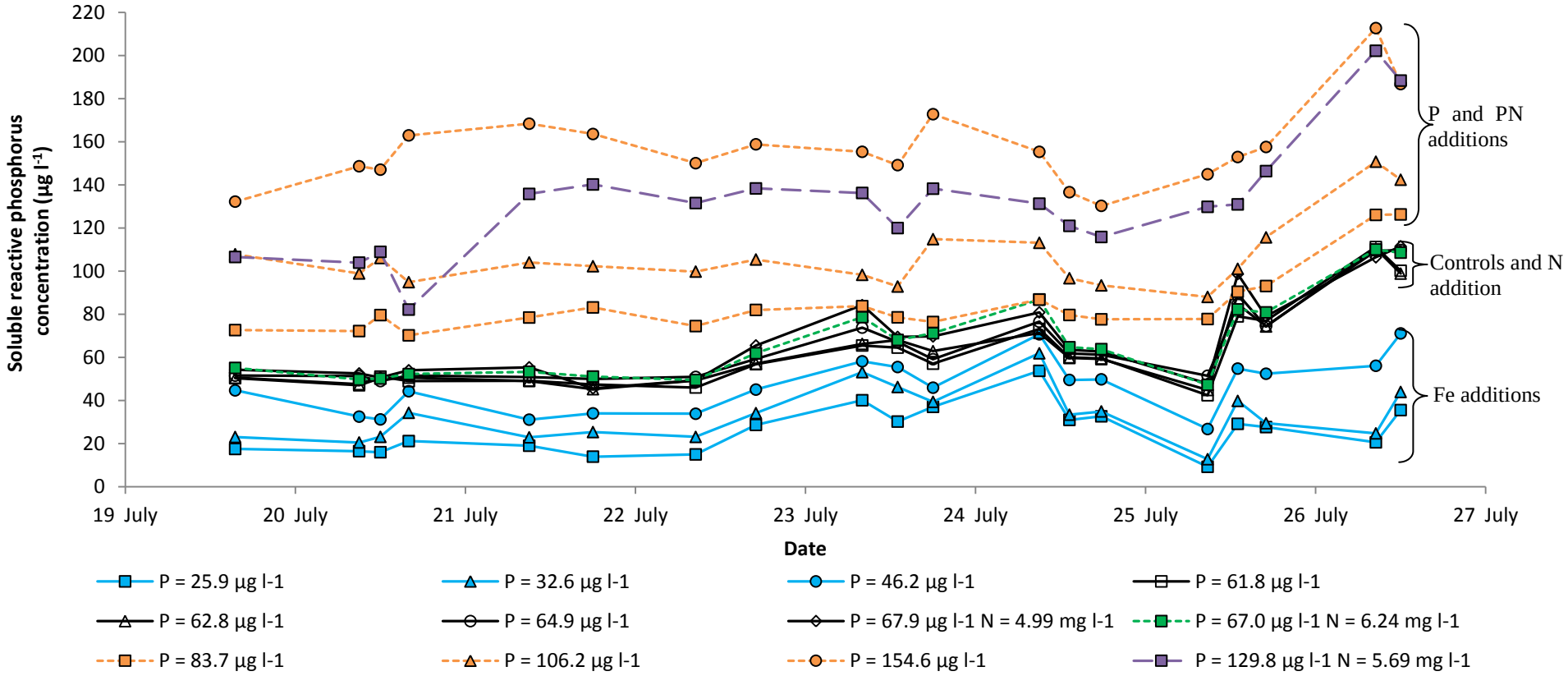


Figure 5.6: Soluble reactive phosphorus concentration in each flume over the course of the seven day nutrient manipulation experiment (Experiment 1). Solid blue line with filled symbol = iron addition (phosphorus reduction), solid black line with open symbol = control (no addition), dotted green line with filled symbol = nitrogen addition (no phosphorus), dotted orange line with filled symbol = phosphorus addition (no nitrogen) and dashed purple line with filled symbol = phosphorus and nitrogen addition.

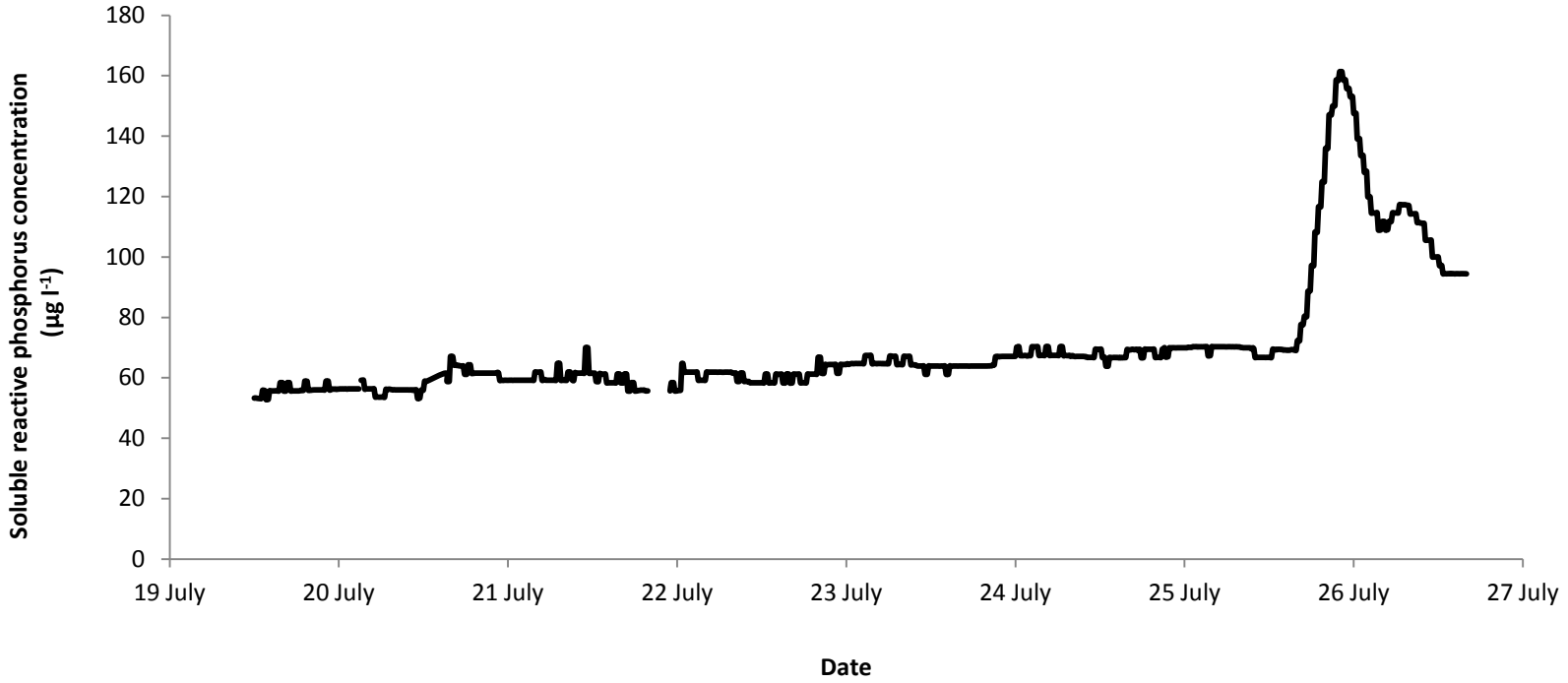


Figure 5.7: Soluble reactive phosphorus concentration in the River Frome at East Stoke (Site 14). Data were measured every 10 minutes for the duration of Experiment 1 by a Phosphax autoanalyser (Hach Lange, Düsseldorf, Germany) (data kindly provided by Wessex Water).

5.3.3 Periphyton biomass response

Based on previous experiments (Bowes *et al.*, 2010, Bowes *et al.*, 2012a, Chapters Three and Four of this thesis), it was expected that the experiment would last approximately 10 days. As a result, intermediate tile substrates were sampled on day three (22nd July 2012) and day six (25th July 2012) with periphyton biomass increasing in all treatments in this time period (Figure 5.8).

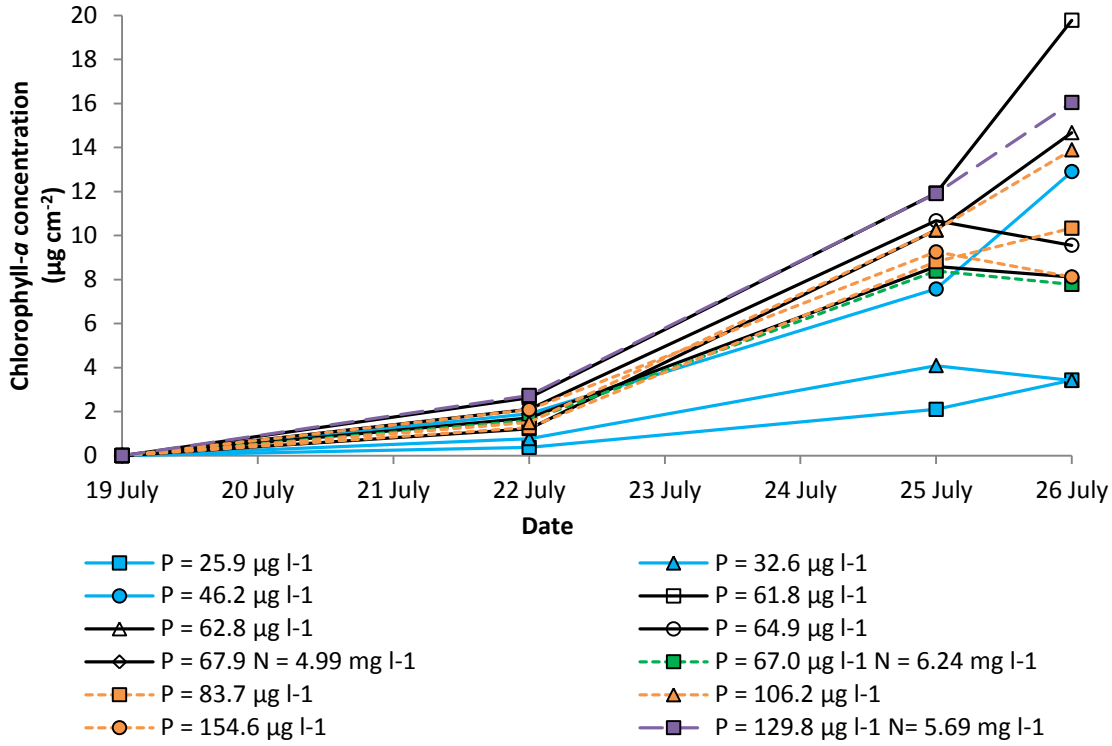


Figure 5.8: Rate of periphyton biomass accrual throughout the experiment. Where biomass has decreased between 25th and 26th July, sloughing of the biofilm has occurred.

Due to the high temperatures and light intensities (Figure 5.5), however, periphyton accrual was much quicker than expected and by day seven (26th July 2012) the biofilms on some of the tiles had begun to slough, as indicated by a decrease in biomass (chlorophyll-*a* concentration) in some of the flumes between the samples collected on day six and day seven (Figure 5.8). As a result, it was decided to stop the experiment and use the samples collected on 25th July 2012 (day six) as the end-

point of the experiment, prior to sloughing, when there were clear visible differences in periphyton biomass between nutrient treatments (Figure 5.9).

Figure 5.9: Periphyton accrual on artificial tile substrates on day six of the experiment in different nutrient treatments. Left = iron addition (soluble reactive phosphorus (SRP) – 25.9 µg l^{-1}), middle = control (SRP – 64.9 µg l^{-1}) and right = phosphorus addition (SRP – 154.6 µg l^{-1}).

Chlorophyll-*a* data were normalised due to the results from Flume 11 (a control flume) being significantly lower than the other controls (Flume 3, 5 and 7) ($F = 8.18$, $P = 0.008$) (Figure 5.10A). After normalisation, a student T-Test revealed significant difference between chlorophyll-*a* concentration and nutrient treatment ($T = 6.76$, $P < 0.001$) (Figure 5.10B). After testing for normality and homogeneity of variance (Bartlett's test statistic = 11.29, $p = 0.934$), a one-way ANOVA revealed significant differences in ash free dry mass (AFDM) value with different nutrient treatments ($F = 51.18$, $p < 0.001$). Post-hoc testing (Tukey's HSD test) revealed that there were no significant differences between control flumes and those in which nutrient concentrations were increased. There were, however, significant differences in AFDM when phosphorus concentrations were decreased (Figure 5.11).

The mean concentrations of chlorophyll-*a* in the control flumes (excluding Flume 11) were 10.24, 10.67 and 10.93 µg cm^{-2} and the mean AFDM in these flumes were

2.50, 2.50 and 2.52 mg cm^{-2}. Increasing SRP concentration from a mean concentration of *ca.* 65 µg l^{-1} to a mean concentration of 154.6 µg l^{-1} had no significant affect on periphyton biomass. The chlorophyll-*a* concentration and AFDM were 10.24 µg cm^{-2} and 2.52 mg cm^{-2} at ambient SRP concentrations (65 µg l^{-1}) decreasing slightly, but not significantly, to 9.25 µg cm^{-2} and 2.42 mg cm^{-2} when SRP concentration was increased to a mean of 154.6 µg l^{-1}.

Increasing mean SRP concentration to what used to be the ambient concentration of the River Frome prior to installation of phosphorus-stripping at Wool STW (106.2 µg l^{-1}) resulted in a chlorophyll-*a* concentration of 10.25 µg cm^{-2} (Figure 5.10A) and AFDM of 2.50 mg cm^{-2} (Figure 5.11). This demonstrates that periphyton communities in the River Frome were not phosphorus limited. Periphyton communities were not nutrient co-limited either, as adding the two nutrients in combination (SRP – 129.8 µg cm^{-2}, NO_3-N – 5.69 mg l^{-1}) resulted in a chlorophyll-*a* concentration of 10.91 µg cm^{-2} and an AFDM of 2.29 mg cm^{-2}.

The three flumes that received iron chloride dosing all accrued significantly less periphyton biomass than the control flumes and those with nutrient additions (Figure 5.10 and Figure 5.11). This demonstrates that ambient phosphorus concentrations in the River Frome are again at the phosphorus-limiting threshold. The amount of periphyton accrued at each reduced SRP concentration was significantly less as SRP decreased. Reducing SRP concentration by 25, 52 and 60 % resulted in accrued chlorophyll-*a* concentration being reduced by 21, 63 and 80 % respectively to mean concentrations of 8.68, 4.09 and 2.10 µg cm^{-2} (Figure 5.10A). AFDM at these reduced SRP concentrations was reduced by 26, 56 and 75 % to values of 1.86, 1.10 and 0.64 mg cm^{-2} respectively (Figure 5.11). These results agree with the visual observations made at the time of the experiment (Figure 5.9).

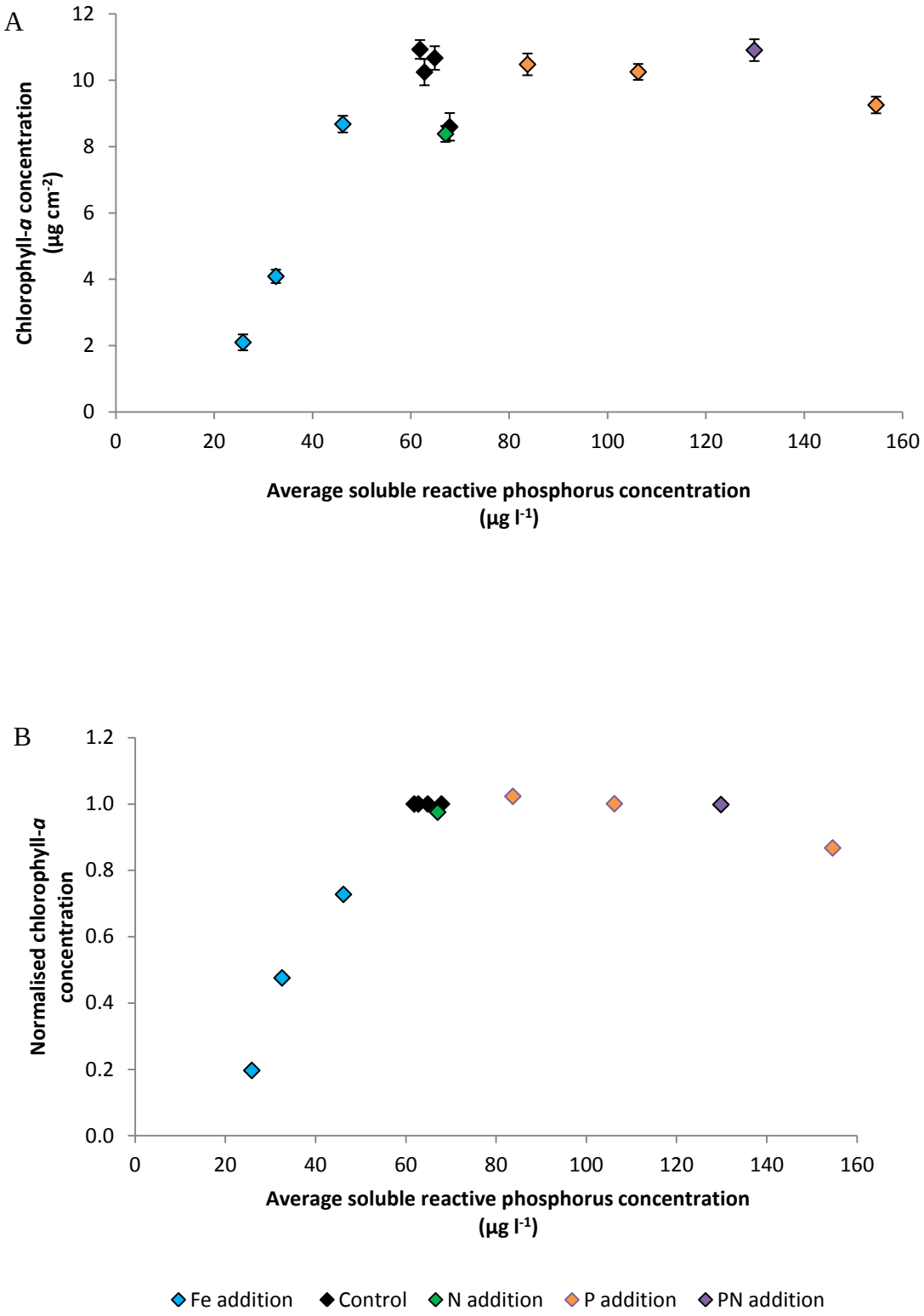


Figure 5.10: Relationship between soluble reactive phosphorus concentration and (A) chlorophyll-*a* concentration after six days (data points are mean values based on analysis of three tiles ± one standard error) and (B) chlorophyll-*a* concentration normalised to the control flume in each set of three flumes.

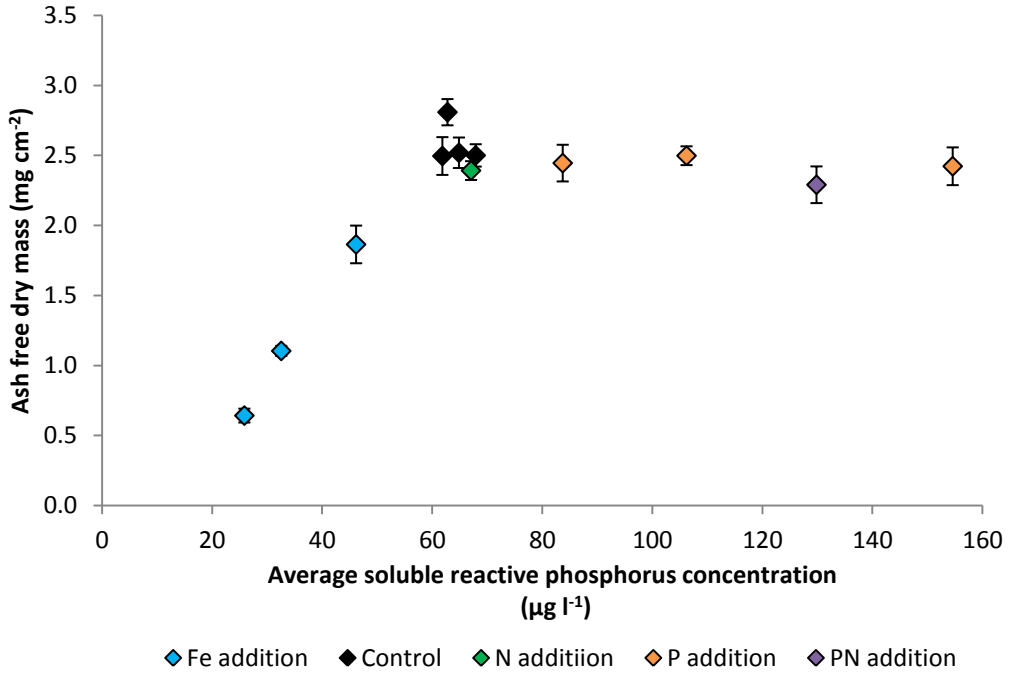


Figure 5.11: Relationship between soluble reactive phosphorus concentration and ash free dry mass after six days . Data points are mean values based on analysis of three tiles ± one standard error.

The curves presented above are similar to those from other rivers where the same flume methodology has been employed (Bowes *et al.*, 2010, Bowes *et al.*, 2012a, Chapter Three of this thesis) and also in other nutrient limitation studies (Dodds *et al.*, 1997, Rier and Stevenson, 2006, Bowes *et al.*, 2007, Suplee *et al.*, 2012). If phosphorus concentration is reduced below the phosphorus-limiting threshold (the break-point of the curve) then, in theory, an ecological effect will be observed (Groffman *et al.*, 2006, Dodds *et al.*, 2010). Bowes *et al.* (2007) suggested that for the River Frome, the phosphorus-limiting threshold was 90 µg l^{-1} (Figure 5.12). However, this experiment has shown that at concentrations of *ca.* 65 µg l^{-1} (the present ambient SRP concentration), adding nutrients had no effect on periphyton biomass. The River Frome was at the phosphorus-limiting threshold in the present experiment (Figure 5.12) and SRP concentrations must be reduced still further in order to observe an improvement in the ecological status of the river.

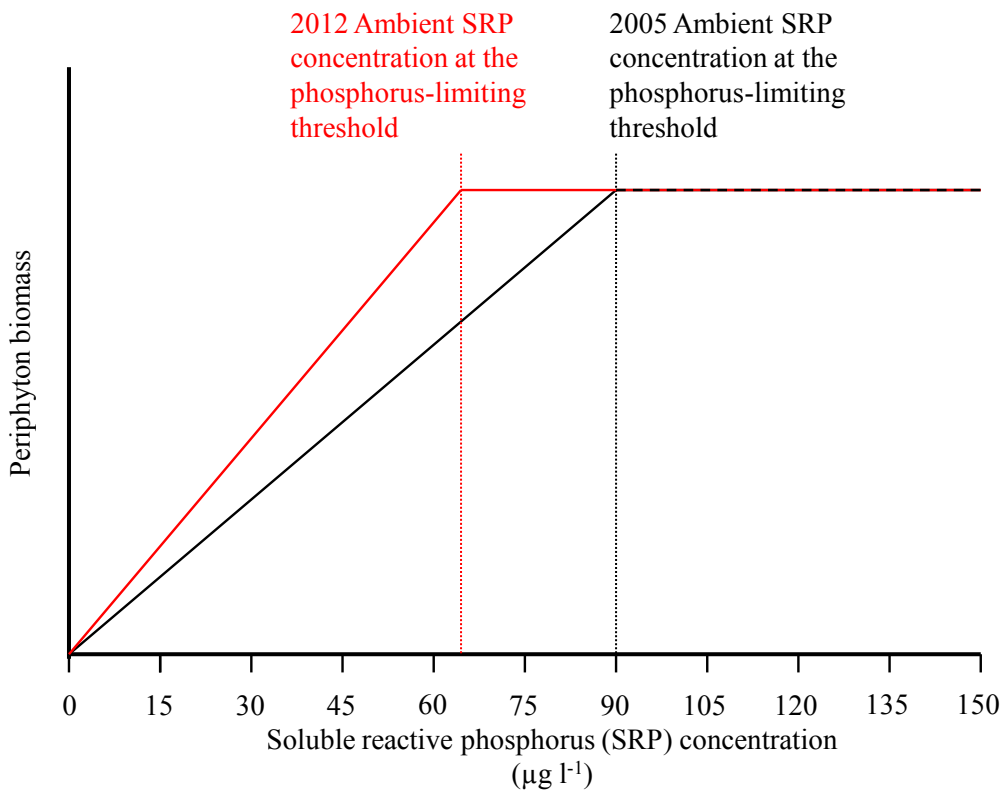


Figure 5.12: Schematic diagram illustrating how the phosphorus-limiting threshold has adapted with changes in ambient soluble reactive phosphorus concentration in the River Frome.

The data presented imply that, since 2005, the phosphorus-limiting threshold has changed and periphyton communities in the River Frome have shifted to be dominated by species that are adapted to lower levels of phosphorus enrichment. This means that the periphyton communities have adapted to the new nutrient environment and are able to accrue maximum biomass at ambient SRP concentrations (65 µg l^{-1}).

Other studies where periphyton have adapted to a new nutrient regime include a long-term direct stream enrichment study undertaken by Peterson *et al.* (1993). Artificial phosphorus enrichment of a tundra stream for four consecutive summers showed that initially there were large increases in periphyton biomass. However, by the third summer, periphyton biomass was suppressed and remained at chlorophyll-*a* concentrations of less than 3 µg cm^{-2} (compared to concentrations of over 20 µg cm^{-2} in the previous two years). This was attributed to ecosystem community adaptation

(Peterson *et al.*, 1993). The current experiment was able to show this process in reverse, with the ecosystem community within the flume biofilms adapting to reductions in phosphorus concentration. The current experiment has shown that any initial reductions in periphyton biomass at the lowered SRP concentration are transient, and the community adapts to the lower phosphorus concentration.

In addition to there being no change in chlorophyll-*a* concentration or AFDM in nutrient treatments greater than the ambient concentration, there was no difference in the autotrophic index (AI) (as indicated by Tukey's HSD post-hoc test). However, reducing SRP concentration caused the AI to increase, representing a more balanced periphyton community with an increase in the proportion of heterotrophs compared to ambient phosphorus and enriched conditions (one-way ANOVA – $F = 31.44$; $p < 0.001$) (Figure 5.13).

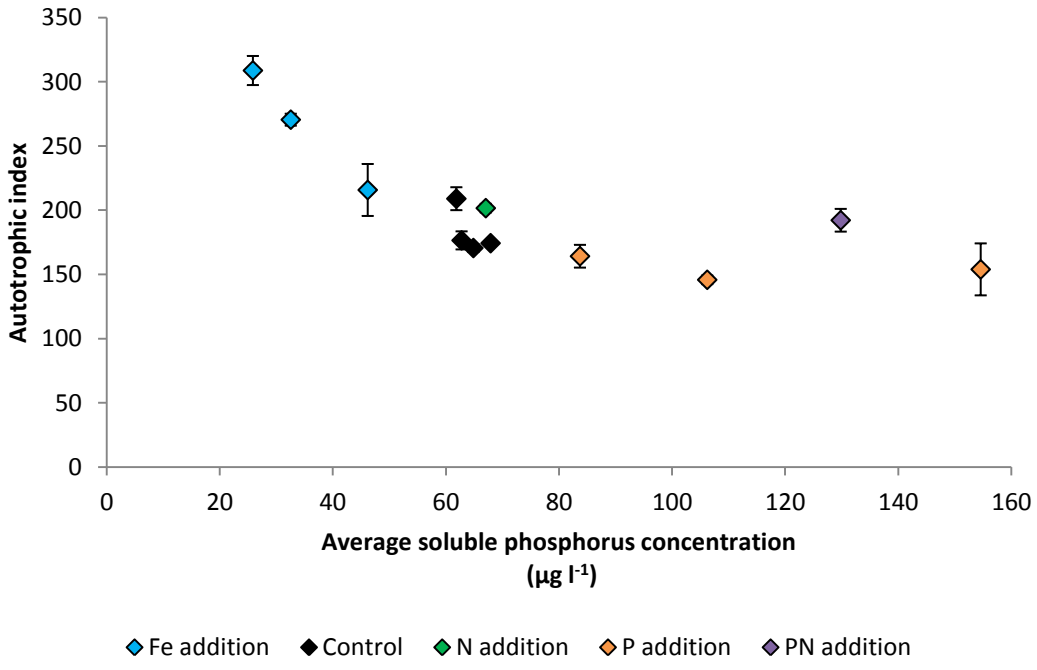


Figure 5.13 : Relationship between soluble reactive phosphorus concentration and the autotrophic index on day six of the experiment. Data points are mean values based on analysis of three tiles ± one standard error.

5.3.4 Diatom assemblages

Diatom assemblages were analysed and the trophic diatom index (TDI) calculated for eight of the 12 flumes (Table 5.3; see Appendix E for names, abundances and sensitivity of all species identified). In a similar way to total periphyton biomass, increasing nutrient concentration had no effect on the TDI with values consistently being between 71 and 74 regardless of increased nutrient manipulation. This indicated a moderate ecological status (UKTAG, 2013b). Reducing ambient SRP concentration to a mean of 46.2 µg l^{-1} throughout the experiment, reduced total periphyton biomass by *ca.* 20 % from a mean chlorophyll-*a* concentration of 10.93 µg cm^{-2} (in the control flume) to a mean chlorophyll-*a* concentration of 8.68 µg cm^{-2} (in the iron dosed flume). However, this 25 % reduction in phosphorus concentration had no effect on the TDI (Table 5.3) with the TDI score remaining at 74.

Table 5.3: Trophic diatom index scores for different nutrient treatments in the River Frome, Dorset.

Treatment	SRP concentration (µg l^{-1})	TDI
Iron addition	25.9	56
Iron addition	32.6	58
Iron addition	46.2	74
Control	64.9	74
Nitrogen addition	67.0	73
Phosphorus addition	106.2	74
Phosphorus and nitrogen addition	129.8	71
Phosphorus addition	154.6	72

When phosphorus concentrations in the River Frome were reduced to 32.6 µg l^{-1}, there was a large decrease in the TDI score, to 58, with a further decrease to 56 when SRP concentration was reduced to 25.9 µg l^{-1} (Table 5.3). At this TDI score, the ecological status of the river would shift from moderate to high (UKTAG, 2013b). This agrees with work of Bowes *et al.* (2012a) in the River Thames which found there to be no change in diatom community structure until SRP concentrations were reduced to *ca.* 30 µg l^{-1}, despite the phosphorus-limiting threshold being defined as *ca.* 100 µg l^{-1}. It also agrees with the findings in Chapter Three of this thesis where (despite a phosphorus-limiting threshold of 45 µg l^{-1}) TDI scores continued to decrease (indicating a better ecological status) until SRP concentrations were below

30 µg l^{-1}. This suggests a lower ecological phosphorus threshold of *ca.* 30 µg l^{-1} may exist if the aim of nutrient mitigation is to promote a better ecological status.

All flumes were dominated by *Cyclotella meneghiniana*, a centric diatom (sensitivity score of 4) that is known to favour higher nutrient concentrations. However, as phosphorus concentrations decreased, so did the proportions of *C. meneghiniana* (Figure 5.14) from percentages of *ca.* 75 % when SRP concentrations were greater than 40 µg l^{-1}, to 43 % when SRP was reduced to an average of 32.6 µg l^{-1}, and 31 % when SRP was reduced to an average of 25.9 µg l^{-1}. Simultaneously, the percentage of nutrient sensitive, araphid, pennate diatoms such as *Synedra ulna* (sensitivity score of 2) increased as nutrient concentrations decreased. At SRP concentrations above 40 µg l^{-1}, *S. ulna* consisted of less than 5 % of the total diatoms identified. This percentage increased to 22 % when SRP was reduced to an average of 32.6 µg l^{-1} and 35 % when SRP was reduced to an average of 25.9 µg l^{-1} (Figure 5.14). This shows it is possible for diatom communities within the periphyton biofilm to adapt to a new nutrient regime over a relatively short time period.

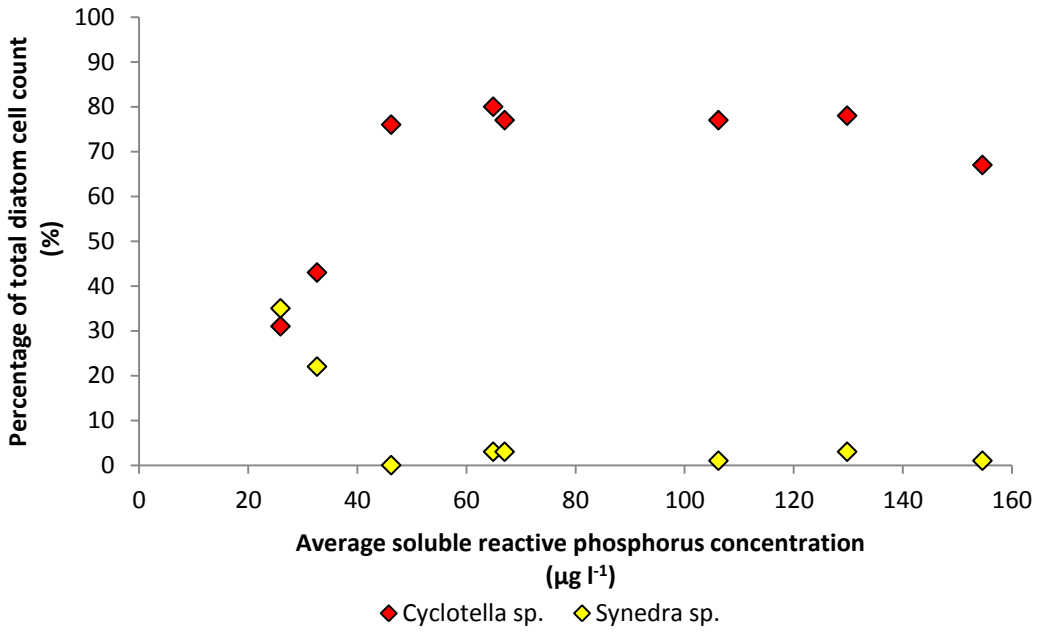


Figure 5.14: Percentage of *Cyclotella* (sensitivity of 4) and *Synedra* (sensitivity of 2) species in the periphyton biofilm according to mean soluble reactive phosphorus concentration.

The TDI scores calculated for the ambient phosphorus concentration and nutrient enriched treatments are comparable with those calculated in diatom surveys conducted by Wessex Water (Cascade Consulting., 2012). The TDI score calculated by Wessex Water for a site at Holme Bridge (approximately 1 km downstream of the flume site with no additional point-source nutrient input) in autumn 2010 was 74. Similarly, TDI scores of the River Frome upstream and downstream of Wool STW were 71.0 and 74.8 respectively in autumn 2010 and 74.3 and 73.7 respectively in autumn 2011. This was despite an increase in mean SRP concentration (sampled on seven occasions) from 60 µg l^{-1} upstream of the STW to 90 µg l^{-1} downstream of the STW (Cascade Consulting., 2012). The lack of significant increase in the TDI despite significant increases in SRP concentration provides further evidence that phosphorus concentrations of 60 µg l^{-1} are above the ecological threshold for diatom community structure in the River Frome.

5.3.5 Periphyton phosphorus content

Unlike the experiment in the River Rede, the periphyton communities in the River Frome did not store excess phosphorus at any of the nutrient treatments examined (Figure 5.15). A one-way ANOVA confirmed that the difference in stored phosphorus between the different nutrient treatments was not significant ($F = 1.91$, $p = 0.089$). This is supported when looking at the biomass response (Figure 5.10 and Figure 5.11). Periphyton communities in all treatments grew and accrued new biomass. As a result, all sequestered phosphorus was being used for life processes associated with photosynthesis and enzyme activity, as well as for growth and reproduction.

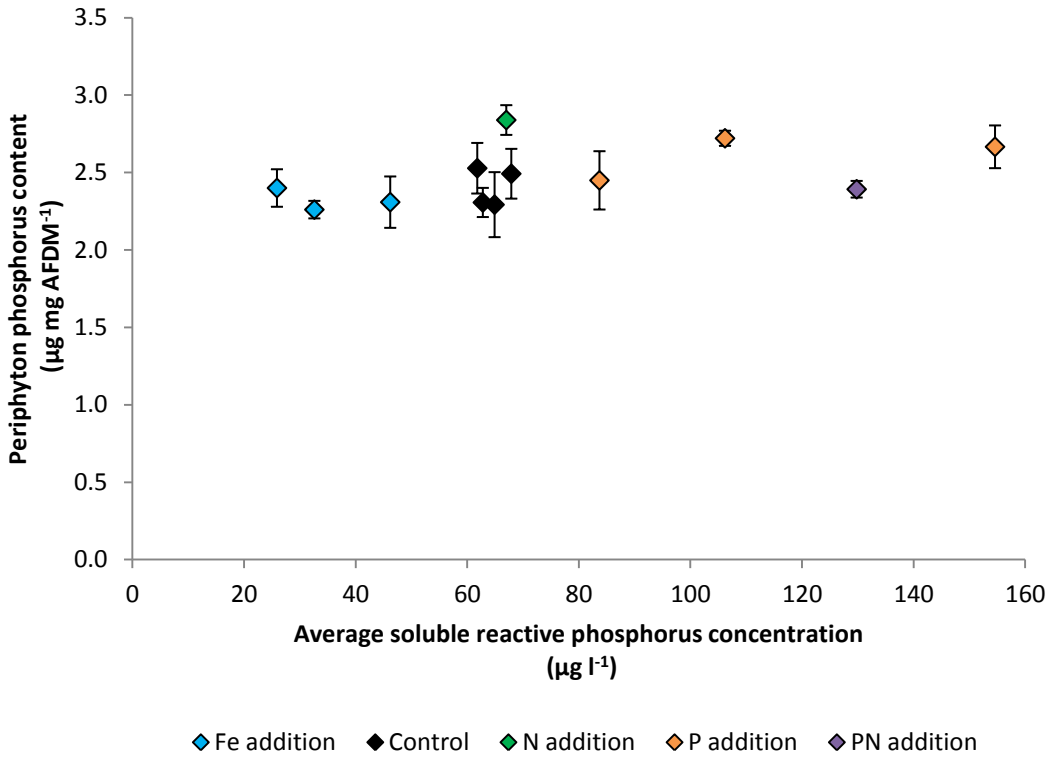


Figure 5.15: Relationship between soluble reactive phosphorus concentration and the periphyton phosphorus content on day six of the experiment. Data points are mean values based on analysis of three tiles ± one standard error.

5.4 Experiment 2 results and discussion

The nutrient concentrations achieved in Experiment 2 (repeat experiment) were all within 14 % of the target concentrations. The mean SRP concentrations (alongside the concentration maintained in the first experiment) are shown in Table 5.4. The concentration of the treatments not receiving phosphorus additions was very similar between the two experiments (less than 3 %). In addition, the flume receiving phosphorus also had near identical mean SRP concentration. The difference in concentration when phosphorus concentration was reduced between the two experiments was more variable; however, the concentrations were similar (Table 5.4).

Table 5.4: Average soluble reactive phosphorus (SRP) concentration at the end of both experiments on the River Frome.

Flume	Average SRP Experiment 2 ($\mu g\ l^{-1}$)	Average SRP Experiment 1 ($\mu g\ l^{-1}$)
7	18.6	25.9
8	154.7	154.6
9	65.3	64.9
10	66.0	67.9
11	26.5	32.6
12	67.9	67.0

Unfortunately, due to a technical malfunction, one of the sets of three flumes (Flumes 1 – 3) sank on day five of Experiment 2 (Figure 5.16). Due to the way the flumes were installed and secured (Figure 5.4), this also disturbed the periphyton biomass in the adjacent set of three flumes (Flumes 4 – 6). Consequently, these experimental treatments were lost and only the results from Flumes 7 to 12 are reported. Light intensities were much lower in the Experiment 2 with a mean maximum light intensity of 71 891 Lx and an average light intensity during daylight hours of 17 834 Lx. Flume water and river temperature between the two experiments was similar with mean temperature of 15.67 ± 3.61 and 15.92 ± 4.79 °C in the flumes and 15.71 ± 3.76°C in the river during Experiment 2 (compared to 16.46 ± 3.20, 16.48 ± 3.42 and 16.12 ± 3.08 °C respectively in the Experiment 1).

Figure 5.16: Photograph of the technical malfunction in flume 1 to 3. The clips holding the float in place snapped causing the flume to sink, disturbing periphyton in the adjacent set of three flumes.

5.4.1 Periphyton biomass response

As a result of lower light intensities, periphyton growth was not as rapid as in Experiment 1. Consequently, the results from day seven of Experiment 2 are reported as there was no evidence of sloughing of the biofilm. Chlorophyll-*a* concentration and AFDM of the periphyton biofilm exhibited the same significant responses as in Experiment 1 (one-way ANOVA: Chlorophyll-*a*: $F = 69.54$, $p < 0.001$; AFDM: $F = 89.15$, $p < 0.001$) (Figure 5.17).

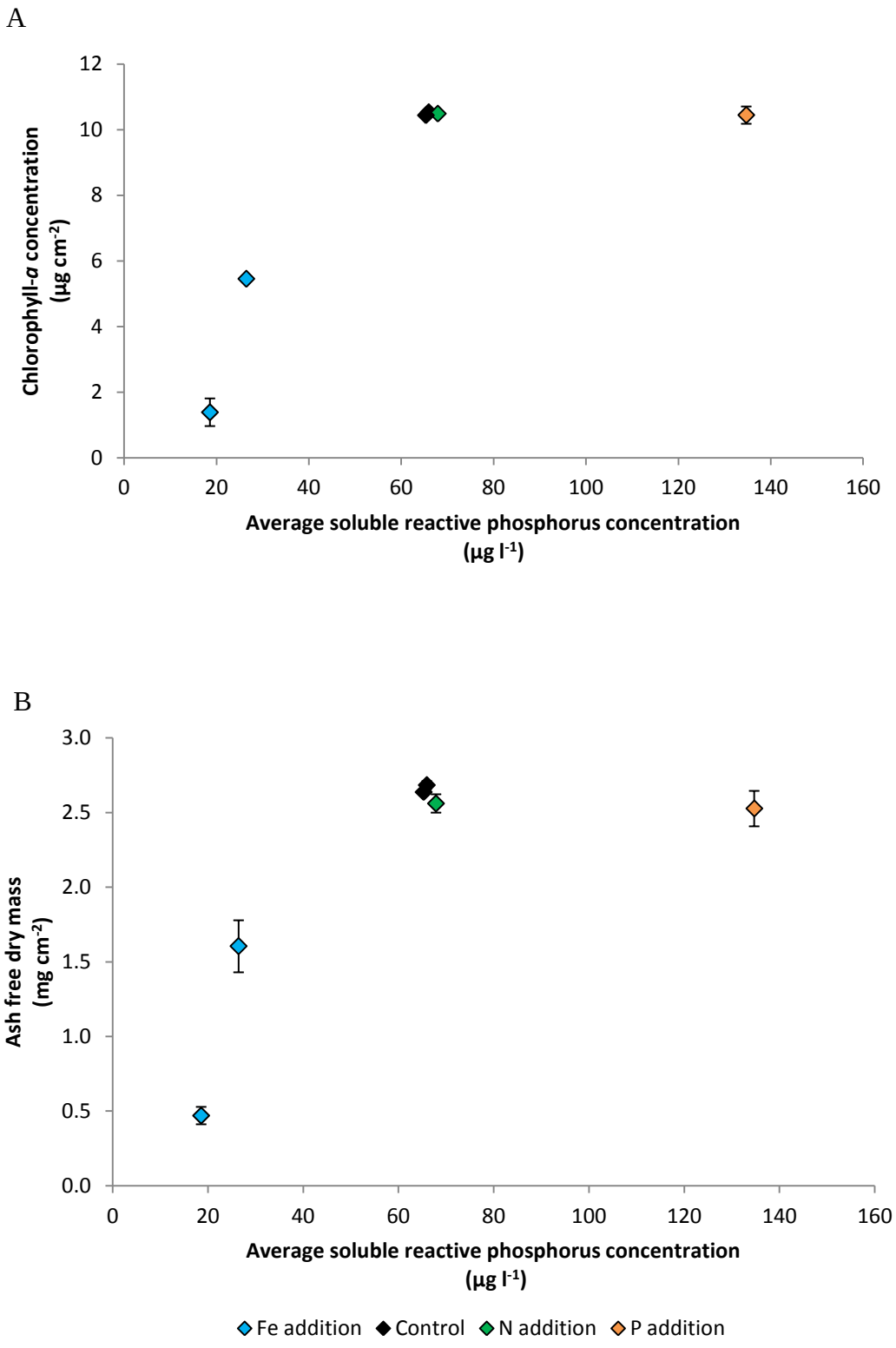


Figure 5.17: Relationship between soluble reactive phosphorus concentration and (A) chlorophyll-*a* concentration and (B) ash free dry mass in Experiment 2. Data points are based on analysis of three tiles ± one standard error.

Post-hoc testing (Tukey's HSD test) confirmed that adding nutrients had no significant effect upon chlorophyll-*a* or AFDM but reducing SRP concentration reduced periphyton accrual rate. Chlorophyll-*a* concentrations were 10.44 and 10.53 µg cm^{-2} in the two control flumes in Experiment 2 compared to 10.24, 10.67 and 10.93 µg cm^{-2} in the control flumes in Experiment 1. For AFDM, values of 2.64 and 2.68 mg cm^{-2} were measured in the control flumes in Experiment 2 compared to values of 2.50, 2.50 and 2.52 mg cm^{-2} in the control flumes in Experiment 1. Increasing and decreasing SRP concentration also resulted in similar results between the two experiments (Figure 5.10, Figure 5.11 and Figure 5.17). This verifies the results and conclusions of Experiment 1, and further confirms that the River Frome is at the phosphorus-limiting threshold.

5.4.2 Experimental reproducibility

The data presented above show good agreement between the results of Experiment 1 and 2. This suggests the two experiments are replicates of each other and the observed responses in periphyton biomass and community composition were as a direct result of nutrient manipulation and not the effects of other confounding factors known to affect periphyton growth (see Section 1.8). To test this further, analysis of covariance (ANCOVA) was undertaken on the slope of the biomass response (chlorophyll-*a* concentration) to see whether or not there was a significant difference in the slopes.

The data points included in the analysis were those from the iron dosed and control flumes. Model II regression (ranged major axis) was run on these slopes to ensure each slope was statistically significant (Table 5.5) before ANCOVA was undertaken. As two pair-wise comparisons were made, a Bonferroni correction was undertaken setting the alpha (significance value) to 0.025. ANCOVA found there to be no significant difference between the biomass response slope in Experiment 1 and Experiment 2 ($F = 4.024$, $p = 0.085$). It can, therefore, be concluded that the two experiments were replicates of each other and the results presented cannot be proved to be different.

Table 5.5: Model II regression analysis of the slopes in chlorophyll-*a* concentration in the iron dosed and control flumes in Experiment 1 and 2 on the River Frome.

Experiment	Statistic	P value	Significant	Regression equation
1	1.299	0.012	Yes	Y = 0.1872X – 1.7778
2	1.008	0.045	Yes	Y = 0.0974X + 0.0966

5.5 River water quality

A longitudinal survey was carried out on five occasions (August 2012, October 2012, December 2012, February 2013 and July 2013) at 14 sites across the Frome catchment (see Figure 5.1 for site locations) in the year following the flume experiment (Appendix F). The aim of this was to put the findings of these flume experiments into context and determine whether at any point the SRP concentration measured in the catchment could be potentially limiting periphyton biomass (i.e. concentration below 65 µg l^{-1}) or could affect diatom community structure (i.e. concentration below 30 µg l^{-1}). The presence of the long-term dataset (Bowes *et al.*, 2011b) also made it possible to compare water quality measured in the survey with that recorded previously. Further data was available for East Stoke (Site 14) as a Phosphax autoanalyser (Hach Lange, Düsseldorf, Germany) was deployed at the site collecting high resolution nutrient data (every 10 minutes). This was maintained by Wessex Water.

The SRP and nitrate data from the longitudinal survey are shown in Figure 5.18. Based on the findings of the flume experiment, this suggests that phosphorus concentrations in Tadnoll Brook (Site 13) could be limiting to periphyton at all times through the year. In addition, phosphorus could be limiting to periphyton growth on the upper and middle reaches of the River Frome (Sites 6, 8, 11 and 12), Wraxall Brook (Site 1), Sydling Water (Site 5) and the South Winterbourne (Site 10) during the summer months. It was during this time that chlorophyll-*a* concentrations of phytoplankton were highest, thus competing with periphyton communities for phosphorus.

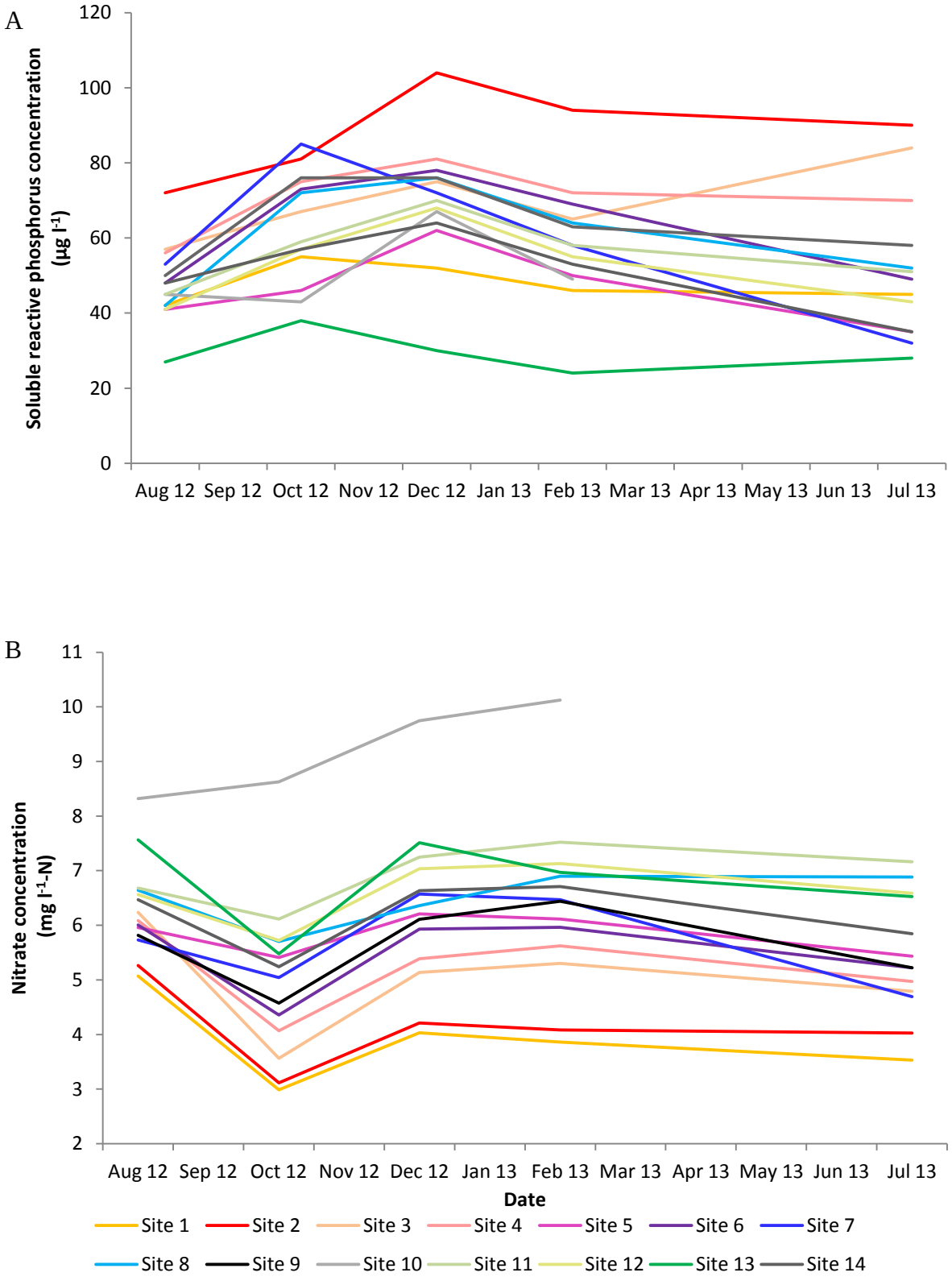


Figure 5.18: (A) Soluble reactive phosphorus and (B) nitrate-N concentration across the Frome catchment between August 2012 and July 2013.

It was only in Tadnoll Brook (Site 13) that present SRP concentrations could potentially affect diatom community composition (i.e. SRP concentrations of less than 30 µg l^{-1} allowing increased abundance of more nutrient sensitive species). This is supported by Wessex Water's TDI analyses. In spring 2010, the TDI was calculated upstream and downstream of the three major STW discharging treated effluent into the River Frome (Maiden Newton, Dorchester and Wool – see Figure 5.1 for location, Sites A, D and F) and the STW at Broadmayne which discharged effluent into Tadnoll Brook (Figure 5.1 - Site E). On the Frome (where SRP concentration was above the phosphorus-limiting threshold) location (i.e. upstream or downstream of the STW input) made no difference to the TDI. However, in the Tadnoll (where SRP was potentially limiting), location did affect the TDI. Values increased from 28.1 upstream of the STW to 49.5 downstream of the STW (Cascade Consulting., 2012). This indicates that the increase in phosphorus concentration as a result of effluent input is affecting the TDI and diatom communities in Tadnoll Brook. Both TDI values for Tadnoll Brook are lower than the Frome, because as the longitudinal survey shows, SRP concentration is lower (and could be truly limiting) in Tadnoll Brook. A TDI of 49.5 is similar to that measured in the flumes (56) when SRP concentrations were reduced to levels similar to that measured in Tadnoll Brook (Table 5.3).

Analysis of Wessex Water's high-resolution phosphorus data (Figure 5.19A) between June 2011 and November 2012 suggests SRP concentrations at the flume site (Site 14 – East Stoke) could be limiting to periphyton growth in October 2011 and March / April 2012. Historically, this was the height of the diatom bloom and coincided with depressions in silicon concentration (Bowes *et al.*, 2011a). Although data from the current longitudinal survey is not at such a high resolution, lowest silicon concentrations were recorded during the February 2013 survey. This implies that periphyton only become phosphorus-limited during large algal blooms.

Bowes *et al.* (2011a) found SRP concentration at East Stoke (Site 14) to be highest from August to October, due to low flows meaning sewage input was not diluted. However, the longitudinal survey found SRP concentrations across the Frome catchment to be highest in December 2012. One reason for this is that flows were artificially inflated in summer 2012 due to high rainfall resulting in dilution of SRP concentration. This was captured in Figure 5.19B which shows the flow (m^{-3} s^{-1}) in

the River Frome at East Stoke (Site 14) in mid-July 2012 was approximately 10-fold higher than what it was in mid-July 2011. An alternative explanation is that due to 'phosphorus–stripping' being installed at Dorchester and Wool STW, sewage effluent was no longer the dominant phosphorus source in the Frome catchment (Bowes *et al.*, 2009a).

Nitrate was the dominant form of nitrogen measured across the Frome catchment. Similar to the findings of Howden *et al.* (2010b) and Bowes *et al.* (2011a), ammonia concentrations were low (typically < 0.5 mg l^{-1}) as (due to the rivers being well oxygenated) any ammonia present was rapidly converted to nitrate. The flume experiments showed nitrogen to be in excess to periphyton biomass accrual at present ambient concentrations at East Stoke (Site 14) of 4.99 mg l^{-1}-N (Figure 5.10 and Figure 5.11). In the River Frome itself, concentrations were only below this value (therefore potentially limiting to periphyton growth) at the most upstream site (Chilfrome, Site 2). The only tributary where values were below this concentration was also in the upper Frome catchment (Wraxall Brook, Site 1). This demonstrates the influence of catchment geology on stream nutrient concentrations. Unlike the majority of the catchment which is underlain by chalk bedrock, the Upper Frome catchment is underlain by Greensand Formations (sandstone) (Arnott *et al.*, 2009), and so receive less nitrogen load from the nitrogen-contaminated groundwater.

When examining the long-term Frome dataset, Bowes *et al.* (2011a) found a seasonal trend in nitrogen concentrations at East Stoke (Site 14) with highest concentrations measured in winter months (December to March) and lowest values being recorded from mid-summer to September, due to uptake and bioaccumulation. This was also the main finding of the analysis conducted by Howden *et al.* (2010b). Disregarding the anomalous result of August 2012 (which was affected by increased surface run-off and abnormally high flows (Figure 5.19B)), this was the case in the present longitudinal survey (Figure 5.18B). The highest nitrate-N concentration recorded in the Frome catchment was 10.12 mg l^{-1}-N in the South Winterbourne (Site 10) in February 2013. All other sites had a peak in nitrogen concentration in December 2012. Water chemistry measurements were not taken on the Frome at Lower Bockhampton (no STW) (Site 8) in February 2013 due to the fast rate of flow, or the South Winterbourne (Site 10) in July 2012 due to drought.

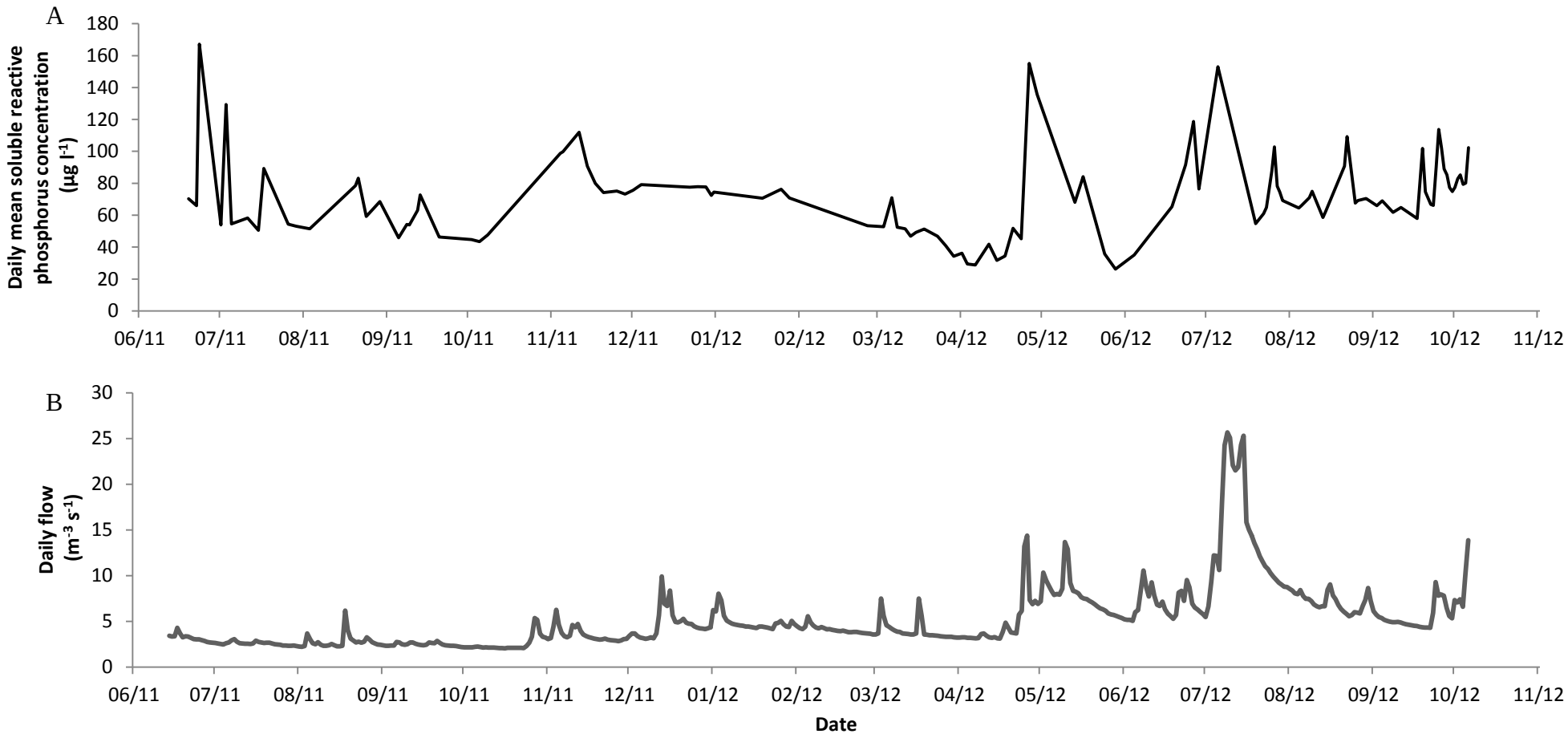


Figure 5.19: (A) Daily mean soluble reactive phosphorus concentration (B) and flow at Site 14 (East Stoke) between June 2011 and November 2012 (data kindly provided by Wessex Water).

5.6 Conclusions

The present study followed on from four previous flume experiments which had concluded that, in moderately phosphorus enriched rivers (40 to 100 µg l^{-1}), the ambient phosphorus concentration is the phosphorus-limiting threshold (Table 5.6). In a similar experiment at the same location, Bowes *et al.* (2007) determined the phosphorus-limiting threshold for the River Frome to be *ca.* 90 µg l^{-1}. As a result of improved sewage treatment, the ambient SRP concentration in the River Frome is now 65 µg l^{-1}. Therefore, the present study repeated the experiment of Bowes *et al.* (2007), using in-stream flume mesocosms to determine whether or not phosphorus concentrations are now truly limiting periphyton biomass accrual in the River Frome.

Table 5.6: Ambient soluble reactive phosphorus concentration in rivers where flume experiments have been conducted previously.

River	Ambient soluble reactive P (µg l^{-1})	Biomass response to nutrient addition	Reference
Kennet	60	No	Bowes *et al.*, 2010
Thames	225	No	Bowes *et al.*, 2012a
Lambourn	45	No	Chapter Three
Rede	15	Yes	Chapter Four

For the first time, the results from the present experiment suggest that it is possible for periphyton communities to adapt to lower phosphorus environments and continue to accrue maximum biomass. Analysis of total periphyton biomass accrual in both Experiments 1 and 2 (Figure 5.10, Figure 5.11 and Figure 5.17) suggests that the current ambient summer SRP concentration of the River Frome (65 µg l^{-1}) is the phosphorus-limiting threshold. Increasing SRP concentrations to those previously determined to be near the threshold (*ca.* 100 µg l^{-1}) had no effect on periphyton biomass. Values of 10.25 µg cm^{-2} and 2.52 mg cm^{-2} were measured for chlorophyll-*a* and AFDM respectively at SRP concentrations of 65 µg l^{-1} (ambient concentration) and 10.24 µg cm^{-2} and 2.52 mg cm^{-2} respectively at the increased SRP concentration (106.2 µg l^{-1}).

From this work, it can be concluded that phosphorus concentrations in the River Frome need to be reduced further in order to see the desired improvement in

ecological status and meet the requirements of the Water Framework Directive. However, it is not possible to determine an exact concentration at which phosphorus is permanently truly limiting and where reductions will not simply result in further periphyton community adaptation. The diatom analysis presented here (and in Bowes *et al., 2*012a and in Chapter Three of this thesis (River Lambourn)) provide some indication of this concentration. The trophic diatom index (TDI) for the River Frome remained high (71 – 74) at all nutrient treatments where SRP was greater than 40 µg l^{-1}. It was only when SRP concentration was reduced to 32.6 µg l^{-1} that there was a significant reduction in the TDI to 58, suggesting a better ecological status. This implies that in order to improve the ecology of the River Frome permanently, the ambient phosphorus concentration needs to be reduced to *ca.* 30 µg l^{-1}. It is at this concentration that there is a change in community structure and communities are, therefore, unable to adapt to accrue maximum biomass at lowered SRP concentrations. This ecological phosphorus threshold agrees with recent thresholds suggested worldwide (Chambers *et al.*, 2012, Suplee *et al.*, 2012) but may be difficult to achieve in UK rivers, which have long suffered the influence of anthropogenic activity.

Chapter 6: Overall discussion of in-stream flume mesocosm experiments.

Chapters Three to Five present the results of individual flume experiments conducted in different rivers across the UK over the summers of 2011 and 2012. Regardless of ambient phosphorus concentration, the minimum chlorophyll-*a* concentration at all sites (*ca.* 2 µg cm^{-2} – non-normalised data) occurred when SRP was reduced below 30 µg l^{-1}. Where SRP concentrations were successfully reduced by the addition of iron chloride, it was possible to perform a series of analyses of covariances (ANCOVA's). This determined whether the difference in slope of the SRP and chlorophyll-*a* concentration relationship for each site was statistically significantly different from each other, taking into account the difference in ambient phosphorus concentrations at the different sites. The normalised chlorophyll-*a* concentrations included in the analyses were those from the iron treated and control flumes (i.e. at SRP concentrations less than the breakpoint in the slope, Figure 6.1). Before each ANCOVA was run, Model II regression was performed to ensure a significant linear relationship in each river (Frome: $p = 0.009$; Lambourn upstream: $p = 0.003$ and Lambourn downstream: $p = 0.022$). For the River Lambourn, chlorophyll-*a* concentrations from unshaded day five treatments were used so that the point in time was comparable to the River Frome. A Bonferroni correction was applied to adjust the significance value and to control Type I error when making multiple comparisons (Quinn and Keough, 2002).

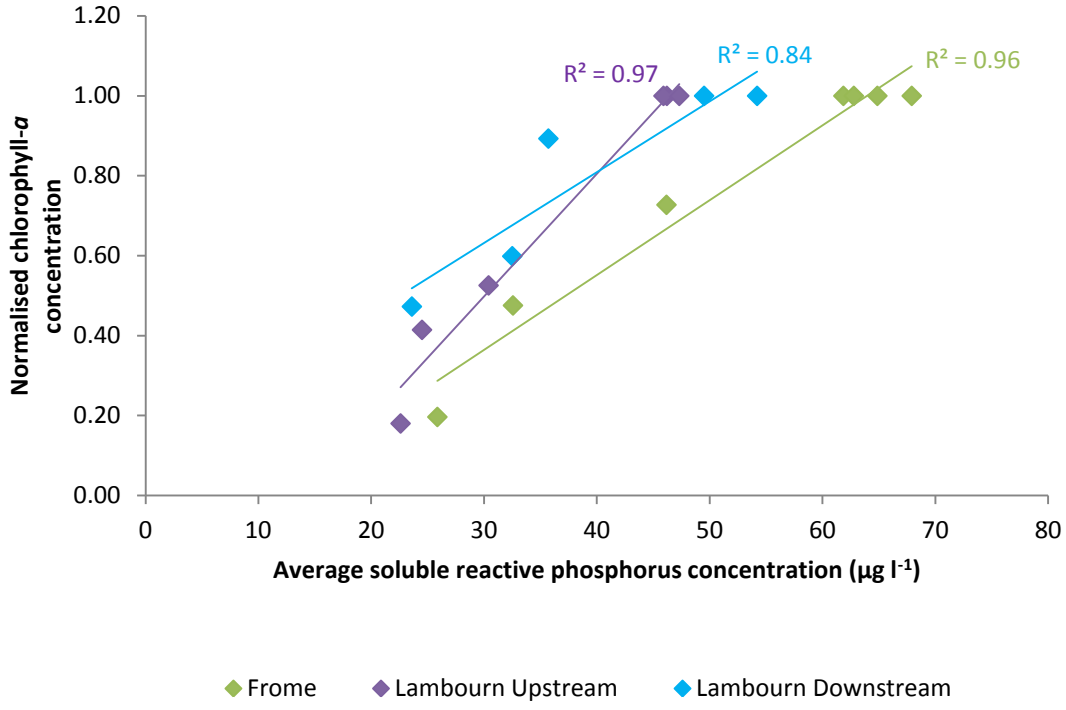


Figure 6.1: Relationship between soluble reactive phosphorus concentration and normalised chlorophyll-*a* concentration in the iron treated and control flumes for sites where the iron-stripping treatment was successful.

The Bonferroni correction adjusted the significance level to p = 0.017. ANCOVA analyses showed there to be no significant difference in slope in the two Lambourn sites (upstream and downstream) ($F = 7.201$, $p = 0.031$) or between the Lambourn downstream site and the River Frome ($F = 0.044$, $p = 0.838$). There was however a significant difference in slope between the Lambourn upstream site and the River Frome ($F = 15.927$, $p = 0.003$). The non-significant relationship between the upstream and downstream Lambourn sites suggests that the STW at Boxford was not significantly affecting the periphyton communities. The results also suggest that periphyton communities in the upstream River Lambourn site were more responsive to increases in phosphorus concentration than the periphyton communities on the River Frome.

The upstream Lambourn site had the lowest ambient SRP concentration (45 µg l^{-1}) so was the closest to the proposed ecological phosphorus threshold of 30 µg l^{-1}. Of the diatom species present at both sites, 49 % had a nutrient sensitivity score of 1 or

2 (highly sensitive) in the Lambourn (upstream) compared to 3 % in the Frome. The communities at the upstream Lambourn site are therefore more sensitive and likely to be less resistant to increasing SRP concentrations than those in the River Frome. The ANCOVA analyses confirm this theory.

Chapter 7: Patterns and causes of stress in phytoplankton across the Thames catchment.

7.1 Introduction

Phytoplankton are free-floating autotrophic micro-organisms including green algae, diatoms and cyanobacteria. Many factors control their growth rate and biomass. These factors fall into four categories: physical constraints (e.g. light intensity and temperature), discharge levels (which affects residence time), chemical measures (e.g. nutrients / land-use) and biological constraints (e.g. grazing / food web short supply, preventing optimum growth being maintained) stress can result. Stress can manifest itself as a decrease in the fluorescence yield of the phytoplankton community (Geider *et al.*, 1993, Graziano *et al.*, 1996, Behrenfeld and Kolber, 1999, Parkhill *et al.*, 2001, Sylvan *et al.*, 2007). If the resource is subsequently supplied in concentrations great enough to alleviate limitation, recovery from stress is indicated by an increase in fluorescence yield (Falkowski *et al.*, 1992).

7.1.1 Fast repetition rate fluorescence (FrrF)

Variable fluorescence methods are able to assess the health of the photosynthetic apparatus within phytoplankton communities and provide an indication of stress by utilising the relationship between chlorophyll fluorescence and photosynthesis. These two processes compete with one another over deactivated excitation energy (Krause and Weis, 1991). One of the most common methods to measure fluorescence is by fast repetition rate fluorescence (FrrF) (Kolber *et al.*, 1998). Fluorescence measurements give important information about the state of the photosystem and the ability of phytoplankton communities to tolerate environmental stress (Maxwell and Johnson, 2000).

Within the photosynthetic apparatus of phytoplankton, light is absorbed by antenna pigments and excitation energy is transferred between the reaction centres of two photosystems, PS I and PS II which are located within the thylakoid membrane of the chloroplasts (Figure 7.1). PS I absorbs light at wavelengths of 700 nm while PS

II absorbs light at wavelengths of 680 nm. At room temperature, the majority of fluorescence is emitted from PS II (Krause and Weis, 1991). PS II is a membrane protein system which harvests light energy to photochemically oxidise water into oxygen (Figure 7.1).

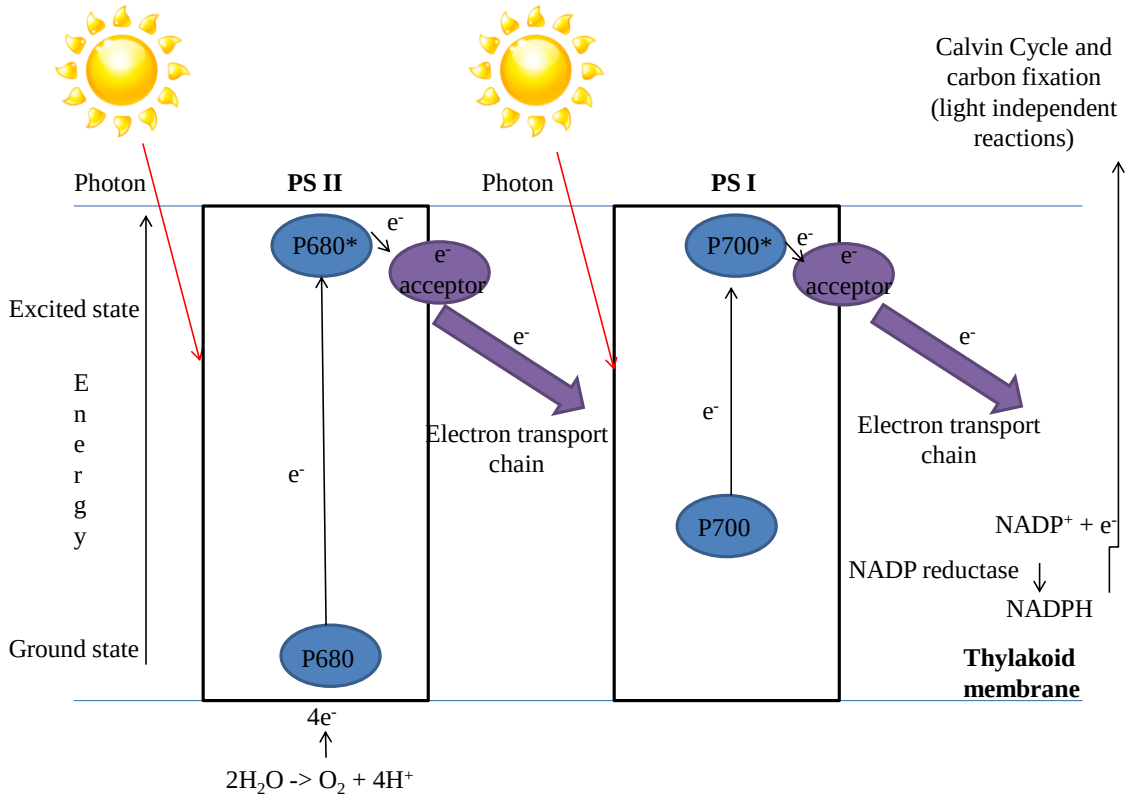


Figure 7.1: Schematic diagram showing the two photosystems (PS I and PS II) within the thylakoid membrane which are involved in the competing processes of photosynthesis and chlorophyll fluorescence.

Under ambient light conditions, all reaction centres within PS II are said to be oxidised (open) with free flow of electrons. Each photon of light absorbed by chlorophyll molecules promotes an electron from the ground state to the excited state and due to the instability of this, on to a primary electron acceptor (plastoquinone – Q_a) (Maxwell and Johnson, 2000). Electrons are then passed along the electron transport chain (ETC) through a series of electron acceptors and the cytochrome b6-f complex (a proton pump moving hydrogen ions into the thylakoid membrane for ATP synthesis) before being passed to PS I. After excitation by a photon of light in PS I (wavelength of 700 nm), electrons are passed to another electron acceptor

(ferredoxin) and on to NADP reductase which uses the electrons to reduce $NADP^+$ to NADPH. This is then transferred to the stroma for use in the Calvin Cycle for ATP production and carbon fixation (Figure 7.1). Under ambient light conditions, the majority of light energy is being used to drive photosynthesis (explained above) so the amount of fluorescence emitted is low allowing the minimum fluorescence (F_o) to be determined (Figure 7.2).

As light intensity increases, the frequency of electron transfer also increases due to light induced activation of enzymes involved in carbon metabolism (Maxwell and Johnson, 2000). Once PS II has absorbed one photon of light and Q_a has accepted an electron, it is unable to accept another until the first has been passed on to a subsequent electron acceptor. During this time, the reaction centre is reduced (closed). As a result, energy cannot be used for photosynthesis, so an increased proportion is emitted as fluorescence (Maxwell and Johnson, 2000), allowing the maximum quantum efficiency of PS II (maximum fluorescence - F_m) to be determined. Instruments used to measure fluorescence are able to provide a saturating flash of light (actinic light) to induce F_m (Figure 7.2).

The difference between the maximum and minimum fluorescence is termed variable fluorescence (F_v) (Figure 7.2) (Krause and Weis, 1991). Variable fluorescence is used to quantify the maximum quantum yield of photochemistry in PS II (fluorescence yield) which is F_v / $F_{m,}$ a measure of the photochemical quantum efficiency. If phytoplankton communities are stressed, a change in the efficiency in either F_o or F_m occurs, resulting in a decreased yield. As such, yield has been recognised to be a sensitive indicator of cell 'health' and stress (Krause and Weis, 1991, Maxwell and Johnson, 2000). Phytoplankton yields under optimum conditions have been determined to be *ca.* 0.65 (Kolber *et al.*, 1998).

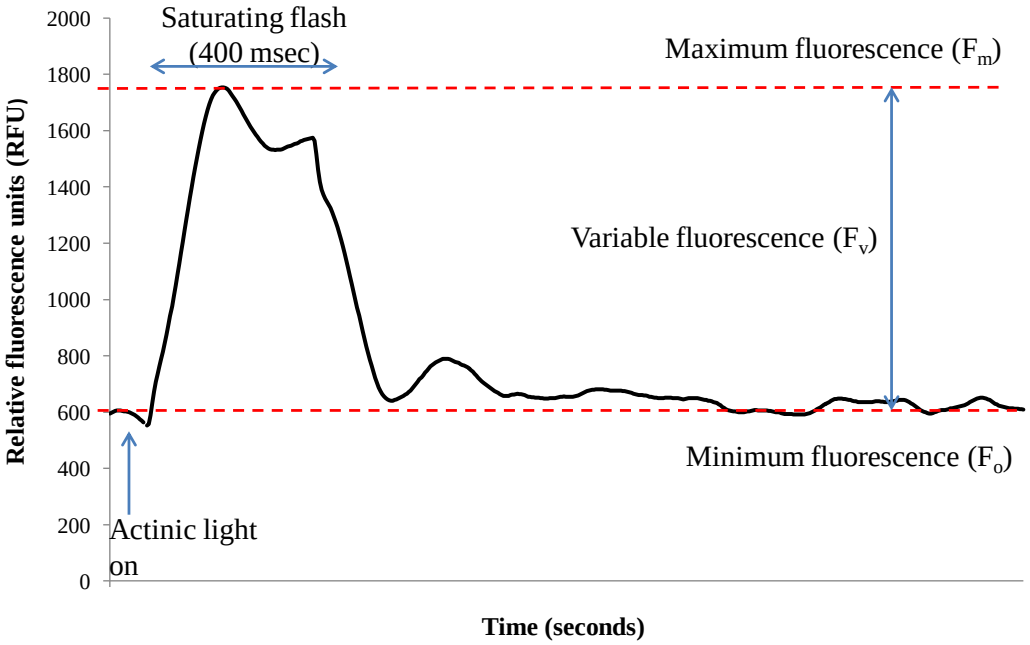


Figure 7.2: Output provided from fast repetition rate fluorometer from which fluorescence yield can be calculated.

Fluorescence has been found to be able to distinguish light limitation from nutrient limitation (Falkowski *et al.*, 1992). To date, studies using FrrF to examine resource limitation of phytoplankton communities have generally been conducted in marine environments (Kolber *et al.*, 1988, Geider *et al.*, 1993, Falkowski and Kolber, 1995, Behrenfeld and Kolber, 1999). The few studies that have occurred in the freshwater environment have been limited to lake ecosystems (Kaiblinger and Dokulil, 2006, Suggett *et al.*, 2006, Kromkamp *et al.*, 2008). Although, Kromkamp *et al.* (2008) were studying a lake ecosystem, their study did include one river sampling site. However, they only sampled on three occasions across one summer with each sampling cruise being just two days in duration. Despite the short duration, the yield of the river site was found to be 0.67 ± 0.027, suggesting phytoplankton communities were not stressed in this case.

7.1.2 Thames catchment description and the CEH Thames Initiative

The River Thames has its source near to the village of Kemble, Gloucestershire and flows in an easterly direction, discharging into the North Sea to the east of London.

To its tidal limit at Teddington, south west London, the River Thames is 354 km in length making it the longest river wholly in England. The total catchment area is 9948 km^2 (Marsh and Hannaford, 2008). Although the catchment includes the UK's capital, London, and other large settlements including Swindon, Oxford, Reading, Maidenhead and Slough (Figure 7.3) the catchment is relatively rural with almost half (45 %) being classified as arable land in the CEH Land Cover Survey (Fuller *et al.*, 2002). A further 34 % of land in the catchment is classified as grassland, 11 % woodland and only 6 % is classified as urban or semi-urban (Fuller *et al.*, 2002).

The CEH Thames Initiative is an integrated monitoring programme which (since February 2009) has monitored river water quality at weekly intervals from 22 sites across the Thames catchment. The sites cover all of the major tributaries as well as six sites on the main stem of the Thames (Figure 7.3). The water quality of these sites ranges from near pristine (River Leach and River Pang), to heavily nutrient enriched (River Thame and The Cut). The monitoring site furthest downstream is the River Thames at Runnymede as, after this site, water quality is affected by tidal influence. Inorganic water quality analysis of the samples includes phosphorus and nitrogen species, dissolved silicon, dissolved organic carbon, major anions and cations, metals, pH, alkalinity, suspended sediment and chlorophyll-*a* concentrations, using the methods described in Section 2.4 (water quality analysis).

Recent years have seen large improvements in water quality across the Thames catchment. These have been largely attributed to reduced phosphorus concentrations in effluent discharge and the introduction of tertiary treatment at STW (Bowes *et al.*, 2010a, Kinniburgh and Barnett, 2010, Bowes *et al.*, 2012a). Despite this, sustained phytoplankton blooms (indicated by peaks in chlorophyll-*a* concentration) are still widespread across the catchment between March and July each year, with many sites experiencing a secondary autumn bloom (August to October) (Whitehead and Hornberger, 1984, Bowes *et al.*, 2012a, Bowes *et al.*, 2012b). However, despite physical and chemical conditions being suited to algal growth, the bloom is not maintained throughout the summer (Waylett *et al.*, 2013). Bowes *et al.* (2012b) have also suggested this could be due to a shift in the phytoplankton community to species dominated by photosynthetic pigments other than chlorophyll-*a* or due to temperature limitation.

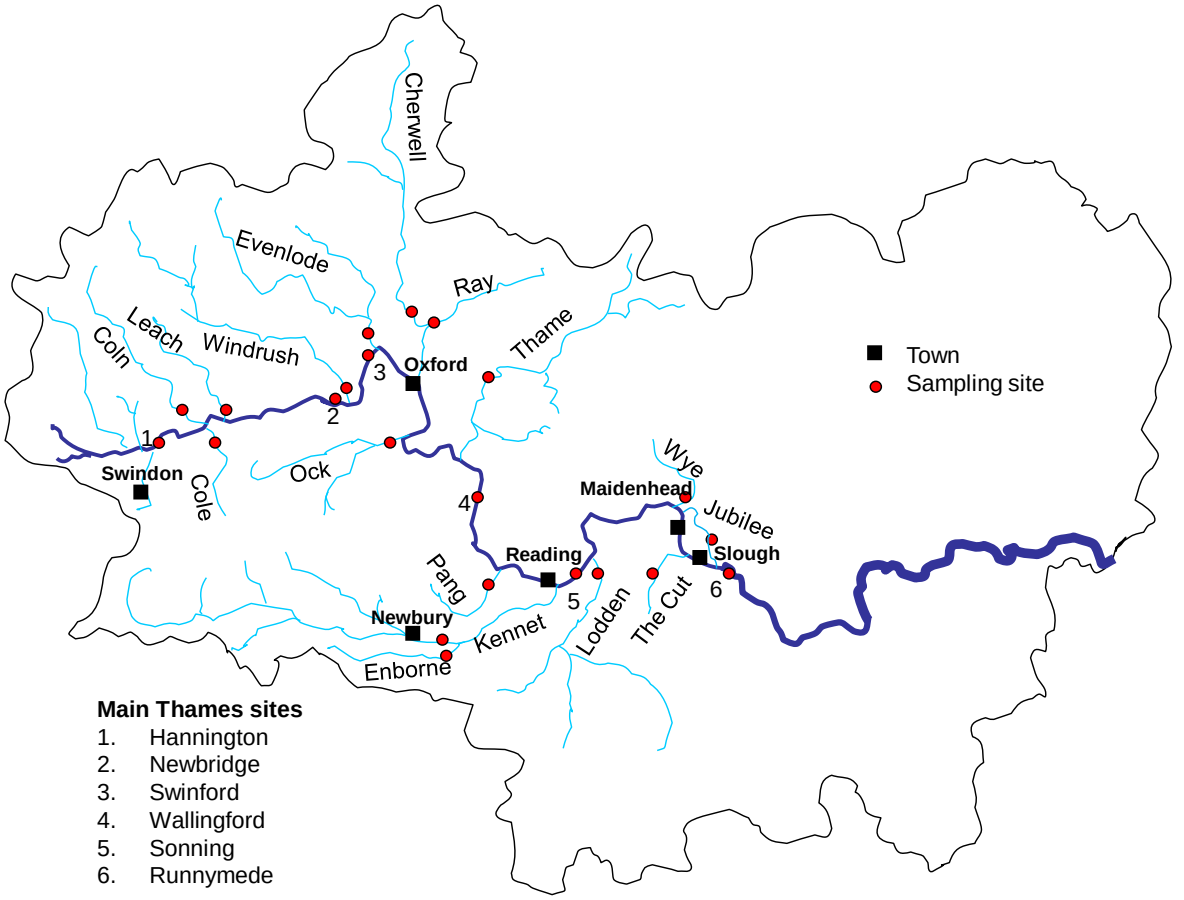


Figure 7.3: Map of the Thames catchment showing the sampling sites on all major tributaries as well as six sites on the main stem of the Thames.

7.1.3 Aim

The use of fluorescence allows a non-invasive, non-destructive, rapid method to monitor photosynthetic events in vivo and allows one to draw conclusions about the physiological state of phytoplankton communities to determine whether or not they are stressed (Krause and Weis, 1991). Therefore, in addition to the standard Thames Initiative samples, a bulk water sample was collected for FrrF analysis between April and July 2013 (see Section 7.2). The patterns in fluorescence yield observed over space and time were investigated and quantified throughout the 2013 algal growing season. This study aimed to utilise the physical and chemical data collected as part of the Thames Initiative to explain patterns in fluorescence yield. Previous chapters in this thesis have shown that ambient nutrient concentrations are rarely limiting to benthic biofilms. This study, therefore, aimed to identify other possible drivers for changing fluorescence yields and attempted to provide an explanation for phytoplankton community stress and the sudden annual collapse of the phytoplankton bloom.

7.2 Methods

The water quality of samples was determined according to the methods described in Section 2.4. Phytoplankton fluorescence yield was determined using a blue PhytoFlash submersible active fluorometer (Turner Designs; Sunnyvale, Canada). This was optimised to examine fluorescence response in chlorophyll-*a* containing functional groups within the phytoplankton community. The 22 sites from across the Thames catchment were sampled at weekly intervals between 8th April and 15th July 2013. Water was collected in a bucket suspended from a bridge so that each sample was collected from the centre of the stream channel at a similar depth (30 cm below the surface). Three water samples were collected at each site, stored upright at room temperature and returned to the laboratory for analysis as soon as practicable (always within six hours).

Once in the laboratory, samples were placed in the dark for 30 minutes to allow all reaction centres within PS II to fully oxidise (open). The PhytoFlash, fitted with a shade-cap to minimise light intensity, was then inserted into the sample and the fluorescence parameters were measured. The PhytoFlash was run in laboratory mode

using the raw fluorescence function so that all data points could be viewed. The algal concentration level was set to 'auto' allowing the instrument to detect the concentration based on the fluorescence signal. A blank sample (0.45 µm filtered river water) was run for each site to allow correction for background fluorescence (i.e. dissolved organic matter and phaeophytin).

When the PhytoFlash was run, three low intensity light emitting diodes (LEDs) were turned on and after a warm up period of 0.5 seconds, 20 fluorescence measurements were determined. The mean of these, minus the blank correction, was the F_o value. Immediately after this, a saturating light from six high intensity LEDs lasting 0.4 seconds was fired (actinic light). During this process, 380 fluorescence measurements were collected. The saturating light flash reduced (closed) the PS II reaction centres and allowed F_m to be determined. F_m was the highest of the 380 fluorescence measurements minus the blank correction. The PhytoFlash also output F_v (determined from $F_m - F_o$) and the fluorescence yield (F_v / F_m). It is the sample yields that will be discussed in detail, as these provide information about stress and the efficiency of phytoplankton photosynthesis.

7.2.1 Effects of temperature and phosphorus concentration

After examination of field data, further laboratory experiments were undertaken to examine the effect of temperature on fluorescence yield. For these, a 25 l bulk river water sample was collected from the centre of the River Thames at Wallingford using a bucket suspended from a rope at 9.00 am on 24th July 2013. The temperature and the initial fluorescence yield of the water were recorded in the field. The water was then transported to the laboratory for SRP concentration to be determined using the method described in Section 2.4.2. In the laboratory, the water was divided across 45 beakers with approximately 300 ± 5 ml in each one. Each beaker was randomly assigned to one of three temperature treatments (10, 15 and 25 °C).

Within each temperature treatment, five nutrient treatments were applied in order to produce a gradient of SRP concentrations. The highest SRP concentration was that of the untreated river water. A concentrated solution of iron (III) chloride was added to the remaining beakers in volumes of 4, 2, 1 and 0.5 ml in order to reduce

phosphorus concentration by precipitation. There were three replicates (A, B and C) of each nutrient treatment, at each temperature.

Three controlled temperature rooms were used for the experiment, set at 10, 15 and 25 °C. Three replicate beakers of each nutrient treatment were placed in each temperature room at 10:30 am on 24th July 2013. Fluorescent lights (Gro-Lux) were set in each room on a 14: 10 hour light: dark cycle. Samples were left for four hours so that water temperature could reach the required temperature. After this, water temperature, fluorescence yield and SRP concentration were recorded for each of the 45 samples. Model II regression (ranged major axis) was run for each temperature treatment to determine whether fluorescence yield was affected by phosphorus concentration.

7.3 Results and discussion

7.3.1 Phytoplankton blooms

As in previous years (Whitehead and Hornberger, 1984, Bowes *et al.*, 2012b), a spring phytoplankton bloom was evident on the middle and lower reaches of the River Thames in 2013. The phytoplankton bloom corresponded with depletion of river SRP concentration between 6th and 20th May 2013 (Figure 7.4). The bloom occurred between 6th May and 3rd June 2103, with a peak at most sites on 13th May 2013. A secondary peak was observed at Runnymede on 1st July 2013. The phytoplankton bloom did not continue throughout July and August (data not shown) despite conditions of light, temperature and nutrients seemingly being favourable to phytoplankton growth. Similar collapses in blooms during mid-summer have been observed in rivers elsewhere (Kromkamp *et al.*, 2008, Neal *et al.*, 2010, Bowes *et al.*, 2011a).

Prior to the bloom (beginning of April 2013), all sites on the River Thames had very similar chlorophyll-*a* concentrations of between 3 and 8 µg l^{-1}. At the peak of the bloom, chlorophyll-*a* concentrations reached 115, 207 and 252 µg cm^{-2} at Wallingford, Sonning and Runnymede respectively. Such high concentrations caused a corresponding depletion in SRP concentration to 37 µg l^{-1} at Wallingford and below the limit of detection (7 µg l^{-1}) at Sonning and Runnymede. After the collapse

of the bloom, SRP concentrations returned to concentrations greater than 200 µg l^{-1} and chlorophyll-*a* concentrations were again similar (less than 21 µg l^{-1}) for all sites on the main Thames (Figure 7.4).

The magnitude of the bloom appears to be closely related to river length, an approximation of residence time (Bowes *et al.*, 2012b). Sites closer to the source (shorter residence time) had less chlorophyll-*a* than those further downstream (Table 7.1). For example, Hannington (46.5 km from the source of the Thames) had a maximum chlorophyll-*a* concentration of 15.3 µg l^{-1} (15th April 2013) with concentrations generally being below 7 µg l^{-1}. Whereas Runnymede (221.7 km from the source), had a maximum chlorophyll-*a* concentration of 252 µg l^{-1} (28th May 2013). In longer rivers, residence times are greater than phytoplankton doubling rates so it is possible for large biomasses of phytoplankton to develop (Hilton *et al.*, 2006). When examining the relationship between maximum chlorophyll-*a* concentration and river length for all 22 sites, a significant linear relationship resulted ($p < 0.001$).

Table 7.1: Changes in the maximum magnitude of the phytoplankton bloom along the River Thames with increasing distance downstream.

Site	Distance downstream (km)	Maximum magnitude of bloom (µg l^{-1} chlorophyll-*a*)
Hannington	46.5	15.3
Newbridge	78.4	19.9
Swinford	89.2	24.3
Wallingford	134.0	114.6
Sonning	166.1	207.2
Runnymede	221.7	252.3

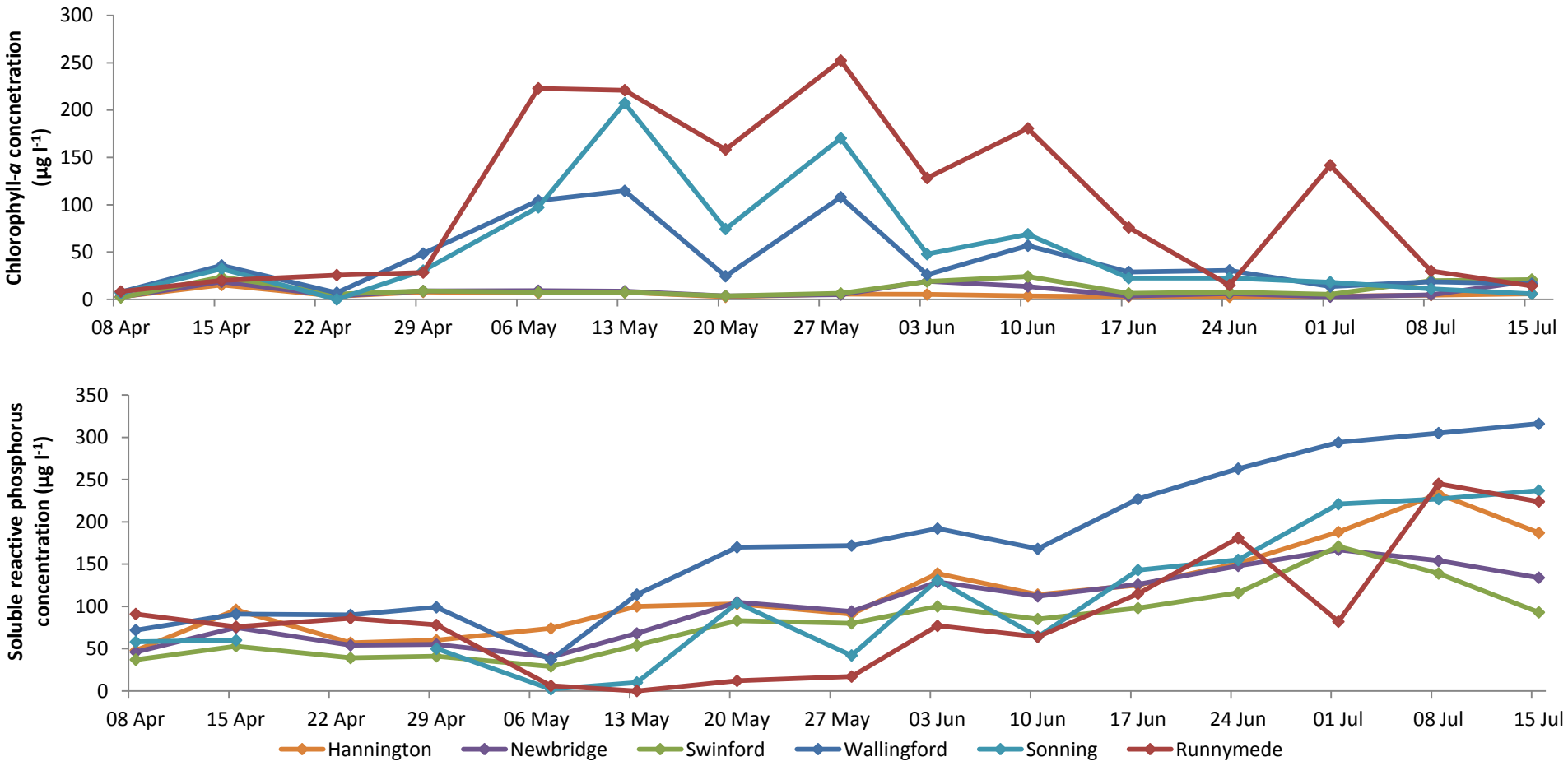


Figure 7.4: (A) Chlorophyll-*a* concentrations and (B) soluble reactive phosphorus concentrations at weekly intervals across six sites on the River Thames between 8th April and 15th July 2013.

7.3.2 Spatial pattern of fluorescence yield

The spatial distribution of fluorescence yield across the Thames catchment at two individual time points, pre-bloom (8th April 2013) and mid-bloom (13th May 2013) are shown in Figure 7.5. Prior to the bloom, yield was greatest (phytoplankton communities least stressed) in some of the tributaries in the mid and lower Thames catchment. Yields were greater than 0.60 in the River Thame, River Ray, River Cherwell, River Evenlode and the Jubilee River. SRP concentrations at these sites at this time point (8th April 2013) ranged from 26 to 213 μg l^{-1}. The lowest yields (phytoplankton communities most stressed) were measured in the tributaries in the rural upper Thames basin including the River Leach, River Coln, River Ock and River Windrush (Figure 7.5). Fluorescence yields at these sites were below 0.35 and SRP concentrations ranged from 8 to 137 μg l^{-1}. The SRP concentration range between the least and most stressed communities were remarkably similar suggesting phosphorus is not the dominant factor controlling phytoplankton stress.

Of the 22 sites studied, the 13th May 2013 marked the peak of the phytoplankton bloom (maximum chlorophyll-*a* concentration) for 12 of the 22 sites. Phytoplankton communities at the five sites that had the highest yield on 8th April 2013 all became increasingly stressed as indicated by decreasing fluorescence yield (Figure 7.5). Yield also decreased on the River Lodden, The Cut, River Enborne and River Thames at Sonning and Runnymede. Conversely, the sites that had the lowest yields pre-bloom all saw phytoplankton communities exhibit less stress (increasing yield) (Figure 7.5). Phytoplankton communities in the River Coln saw the largest increase in yield between the two time points (0.343 on 8th April 2013 to 0.574 on 13th May 2013) while the communities in the River Thames at Runnymede exhibited the largest decrease in yield (0.583 to 0.314). The SRP concentrations between the two time points decrease from 27 to 21 μg l^{-1} in the River Coln, suggesting phosphorus concentrations were not the dominant factor in regulating yield at this site. In the River Thames at Runnymede, the decrease in yield occurred at the same time as SRP concentrations were reduced from 91 μg l^{-1} on 8th April 2013 to below detection limits on 13th May 2013. In this case, it was plausible that the increase in phytoplankton community stress was a direct result of phosphorus depletion.

The observed spatial patterns in yield are similar to those observed in chlorophyll-*a* concentration by Bowes *et al.* (2012b) and can be attributed to residence time (see Section 7.3.3). On the River Thames itself, it was the sites further downstream (longer residence time to allow phytoplankton bloom development) that had the highest maximum chlorophyll-*a* concentrations (Table 7.1). Those same sites (Sonning and Runnymede) also saw the largest decreases in fluorescence yield at the height of the phytoplankton bloom. In addition to this being related to depletion in phosphorus concentration, it was possible that the increase in stress was a result of self-shading within the phytoplankton community and competition for light (Whitehead and Hornberger, 1984).

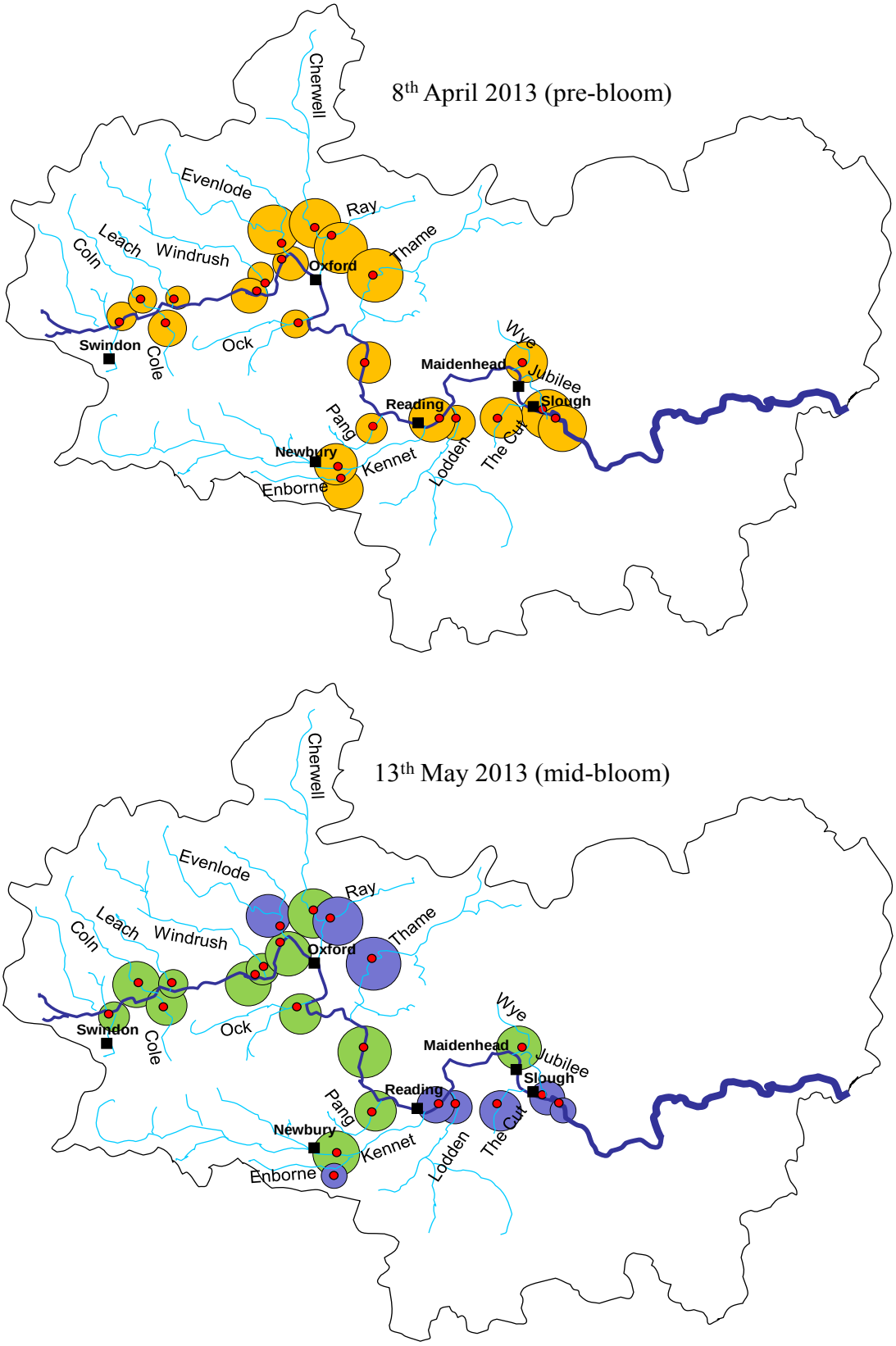


Figure 7.5: Changes in fluorescence yield across the Thames catchment prior to and during the phytoplankton bloom.Bigger circles = higher yield. Orange = background yield, purple = decrease in yield from 8th April and green = increase.

7.3.3 Effect of river length

Distance to source (i.e. river length) was calculated using geographical information system (GIS) software and CEH's Intelligent River Network (Dawson *et al.*, 2002). In general, rivers with a smaller distance to source (i.e. shorter rivers) had lower yields and phytoplankton communities were more stressed compared to those with a greater distance to source (i.e. longer rivers) (linear regression $p = 0.008$) (Figure 7.6). Rivers over 80 km in length all attained background yields similar to what Kolber *et al.* (1998) defined as the optimum yield representing healthy, non-stressed phytoplankton communities (0.65). Whereas shorter rivers, such as the River Enborne (25.5 km), only attained a background yield of 0.315 with a maximum yield of 0.486 and a minimum yield of 0.104. As well as being more stressed, shorter rivers generally had lower chlorophyll-*a* concentrations compared to longer rivers. The exception to this was the River Ray which generally had chlorophyll-*a* concentrations below 45 µg l^{-1} but experienced a phytoplankton bloom where chlorophyll-*a* concentrations peaked at 111.55 µg l^{-1} on 28th May 2013.

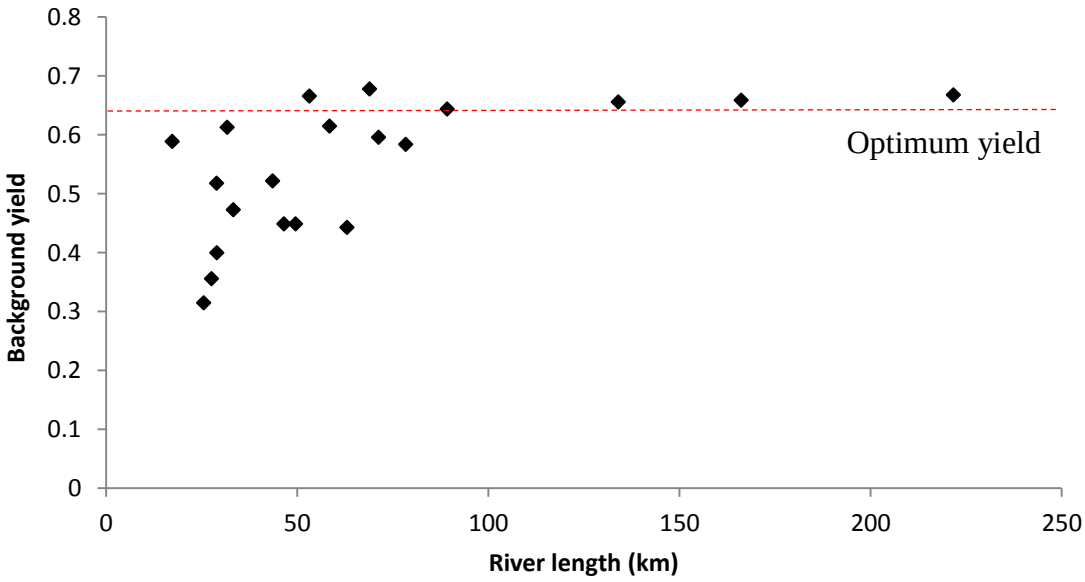


Figure 7.6: The relationship between background yield and river length. Red dashed line represents the optimum yield (0.65) as defined by Kolber *et al.* (1998).

Some of the sites showed great variation in yield throughout the 15 week monitoring period, while others had a near constant yield. The sites with a relatively constant background yield (the River Cherwell, the River Kennet and the River Thames at Newbridge, Swinford and Wallingford) all showed no signs of stress with yields ranging from 0.584 to 0.678 throughout the monitoring period. These sites ranged in length from 68.9 to 134.0 km so were around the distance threshold between stressed and non-stressed phytoplankton communities (80 km), based on Figure 7.6.

River length was the dominant factor in controlling whether the background fluorescence yield followed or was the inverse of the relationship with chlorophyll-*a* concentration. For the majority of rivers below 100 km in length, yield generally followed the same pattern as chlorophyll-*a* concentration, so yield increased as chlorophyll-*a* concentration increased and vice versa (Figure 7.7). This suggests that phytoplankton growth rate was highest when physical and chemical conditions were most favourable, and phytoplankton stress was at a minimum. The photosynthesis process is outcompeting fluorescence over excitation energy in PS II (Section 7.1.1). Across the Thames catchment, there were some tributary sites (the River Wye, River Ock, River Ray and River Cherwell) which had no discernible pattern. However for the longest rivers, the River Thames at Sonning (166.1 km), the Jubilee River (209.2 km) and the River Thames at Runnymede (221.7 km), yield decreased as chlorophyll-*a* increased (Figure 7.7). As well as this pattern being related to river length, it was also due to magnitude of phytoplankton bloom. These three rivers all had peak chlorophyll-*a* concentrations greater than 200 µg l^{-1}. This suggests that phytoplankton communities were stressed at such high chlorophyll-*a* concentrations possibly due to self-shading within the phytoplankton community, or due to other resources (such as bioavailable phosphorus or silicon) becoming depleted due to rapid phytoplanton growth rates. At the onset of phosphorus depletion due to high phytoplankton biomass, fluorescence yield remained high for a week before communities showed signs of stress (Figure 7.7 – Thames at Runnymede; 7th and 13th May 2013). It is plausible that during this lag-period, fluorescence yield was being maintained due to phytoplankton utilising stored phosphorus within their cells.

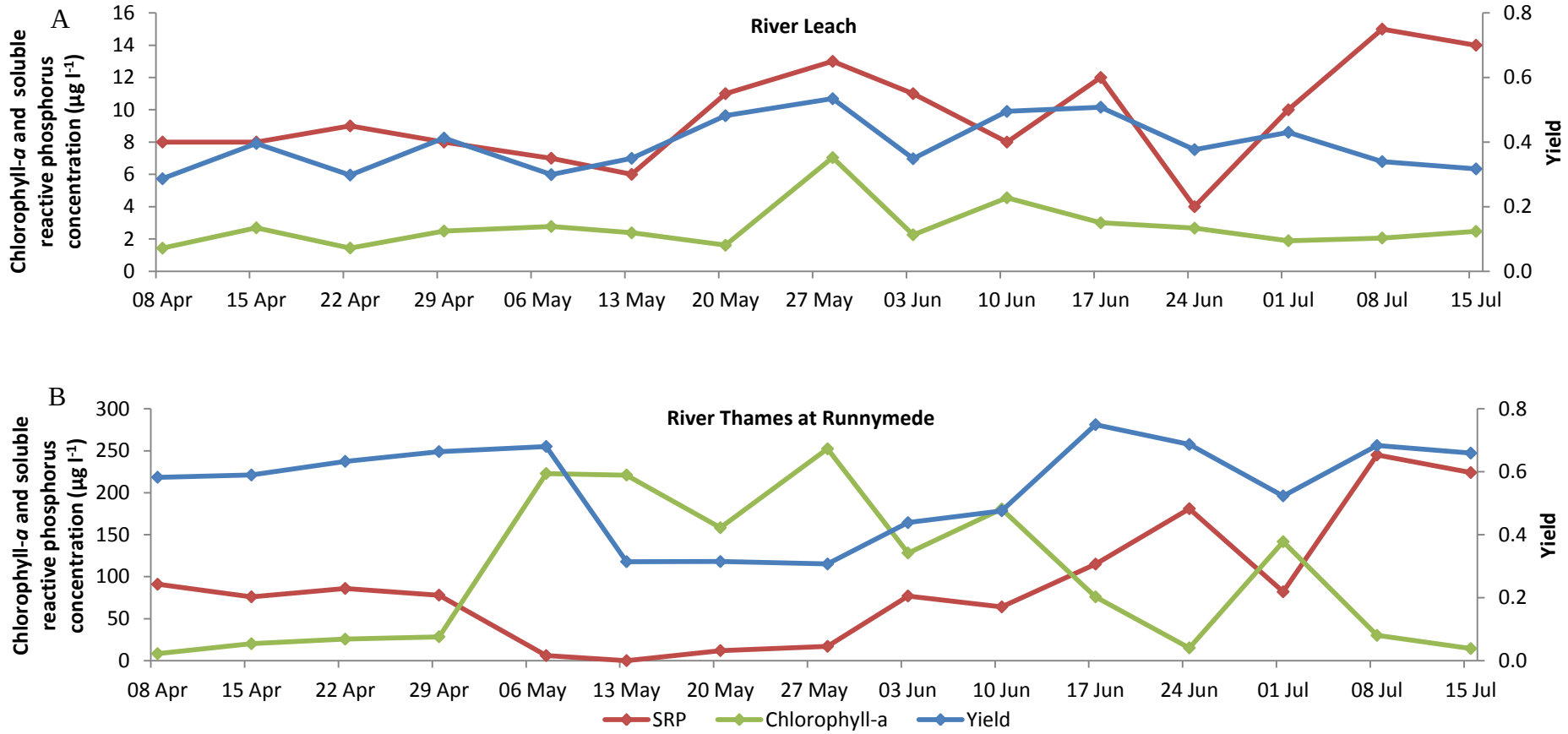


Figure 7.7: Relationship between chlorophyll-*a* concentration and yield in (A) the River Leach (mean SRP = 10 µg l^{-1}, maximum chlorophyll-*a* = 7.04 µg l^{-1}) where the yield follows the chlorophyll relationship and (B) the River Thames at Runnymede (mean SRP = 90 µg l^{-1}, maximum chlorophyll-*a* = 252.31 µg l^{-1}) where the pattern in yield is the opposite to that in chlorophyll. Soluble reactive phosphorus concentration follows the same pattern as yield in both rivers.

7.3.4 Effects of phosphorus concentration

Previous work examining benthic algae in the River Thames has concluded that the phosphorus-limiting threshold for this river was *ca.* 100 µg l^{-1} (Bowes *et al.*, 2012a). The results presented so far in this thesis (Chapters Three to Five) have shown that in addition to the phosphorus-limiting threshold, rivers also exhibit a lower ecological threshold of *ca.* 30 µg l^{-1}. Regression analyses between bioavailable phosphorus (SRP) concentration and yield across all rivers within the Thames catchment pre-bloom (8th April 2013) and mid-bloom (13th May 2013) were not significant (pre-bloom $p = 0.150$, mid-bloom $p = 0.183$) suggesting phosphorus was not the main variable controlling the stress response of phytoplankton and was not limiting to river ecology.

It was expected that the phytoplankton communities at sites with an annual average phosphorus concentration below 100 µg l^{-1} (the proposed phosphorus-limiting threshold) would exhibit signs of stress, as indicated by a lower fluorescence yield. The four rivers in the Thames catchment with annual total phosphorus concentration below 100 µg l^{-1} were the River Leach and River Coln in the upper catchment and the River Pang and River Kennet in the middle of the catchment. The lengths of these tributaries ranged from 27.6 km (River Pang) to 71.3 km (River Kennet). If fluorescence yield was affected by phosphorus concentration, it would be expected that yield would increase with increasing phosphorus concentration, as the phosphorus concentrations can be assumed to be truly limiting to phytoplankton (as they are below the threshold). Of the 22 sites examined, it was only in the River Leach that phosphorus concentration was similar to the 30 µg l^{-1} ecological threshold identified in Chapters Three to Five.

Figure 7.8 shows phosphorus to have no relationship with fluorescence yield in the four rivers with annual total phosphorus concentrations below 100 µg l^{-1}. The Leach had the lowest annual total phosphorus concentrations (31 µg l^{-1}), yet for over half of the monitoring period, it had one of the highest yields. The maximum yield of 0.534 was comparable with that measured on the River Lodden (0.554) which had a much greater phosphorus concentration (annual average total phosphorus concentration – 213 µg l^{-1}). This agrees with the work of Kromkamp *et al.* (2008), who found low external phosphorus concentration did not result in lowered yields. Conversely, the

River Pang had an average annual total phosphorus concentration of 66 µg l^{-1} but the yield from 20th May 2013 onwards was the lowest of the four low phosphorus sites. This was after the phytoplankton bloom and could be a result of depletion of internal phosphorus stores within the phytoplankton cells (Kromkamp and Peene, 1999). The fact that phosphorus is not controlling phytoplankton community stress below the apparent phosphorus-limiting threshold provides evidence for a (lower) ecological phosphorus threshold. Further evidence to support a lower ecological threshold is provided when examining the yield response in large rivers where phosphorus was depleted at the height of the phytoplankton bloom, for example, the River Thames at Runnymede. As Figure 7.7B shows, yields remained high when SRP was high but as SRP was depleted and concentration dropped below 30 µg l^{-1}, phytoplankton communities became increasingly stressed. The lowered yield was sustained for a period of five weeks and yield only recovered when the phosphorus concentration returned to above 30 µg l^{-1}.

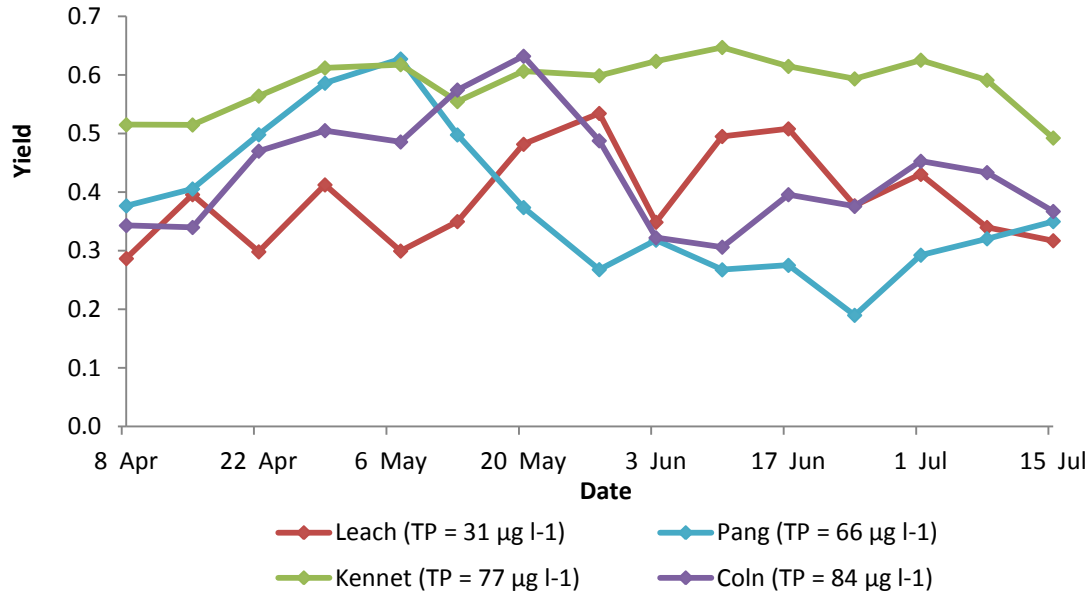


Figure 7.8: Patterns in yield throughout the 15 week monitoring period at sites where annual average phosphorus concentration is below 100 µg l^{-1}.

Much river restoration and phosphorus mitigation work has been undertaken on the River Kennet, with phosphorus-stripping being undertaken at all major STW (Neal *et*

al., 2010). Bowes *et al.* (2012b) present strong evidence to suggest reduced phosphorus concentrations were beginning to limit phytoplankton blooms on this river. However, the fluorescence yields presented in Figure 7.8 (0.515 to 0.647) suggest the phytoplankton communities were not stressed. Chapter Five of this thesis concludes that at SRP concentrations greater than 30 μg l^{-1} (the proposed ecological phosphorus threshold), any beneficial ecosystem effects observed as a result of phosphorus reduction are only temporary. Ultimately, the (benthic) algal community will adapt to produce maximum biomass at the lowered phosphorus concentration. The results presented suggest the same is occurring in the phytoplankton community of the River Kennet. A marine based study by Parkhill *et al.* (2001) also found that nutrient limitation did not always affect fluorescence yield. In environments with stable nutrient concentrations (as in the River Kennet), Parkhill *et al.* (2001) found long-term acclimation could result in recovery of fluorescence yield. Yields, therefore, were the same as in nutrient replete environments.

7.3.5 Best subsets regression

As shown in Section 7.3.3 and 7.3.4, fluorescence yield was affected by river length and not by phosphorus concentration. Best subsets regression was applied to the whole dataset in Minitab (version 15) to determine which variables produced the strongest model to predict fluorescence yield. Best subsets is a method that determines which variables should be included in a multiple regression model and examines all the models created from a set of variables (predictors) to produce the strongest fit (highest R^2 value).

Adding river length to the annual average phosphorus concentration regression model increased R^2 from 7.6 to 49.9 and adjusted R^2 from 3.0 to 40.5. The regression equation for this model is given in Equation 6.1.

$$\text{Yield} = 0.401 + 0.000242 * \text{TP} + 0.00123 * \text{Length}$$

[Equation 7.1]

Adding silicon as a third variable increased R^2 by a similar amount (from 7.6 to 54.7). The addition of maximum chlorophyll-*a* concentration, temperature, suspended sediment and carbon concentration further improved the R^2 and adjusted

R^2 to 73.7 and 63.2 respectively. The number of determinants involved in increasing the R^2 value to greater than 70 illustrated the complex interrelated nature of variables controlling fluorescence yield.

As river length was determined to interact with phosphorus concentration to determine fluorescence yield, the effects of phosphorus concentration were examined by looking specifically at yield response in rivers of a similar length (30 ± 3.5 km). A regression analysis was run on the relationship between yield and average annual phosphorus concentration in these (Figure 7.9). The rivers included were the River Pang (27.6 km), River Cole (28.9 km), River Leach (29.0 km), River Ray (31.7 km) and River Ock (33.3 km). The linear regression was found to be significant ($p = 0.011$) with a R^2 value of 0.92. Background yields increased from 0.400 and 0.356 in the River Leach and Pang (annual average total phosphorus concentration of 31 and 66 µg l^{-1} respectively) to 0.613 in the River Ray (annual average total phosphorus concentration of 487 µg l^{-1}).

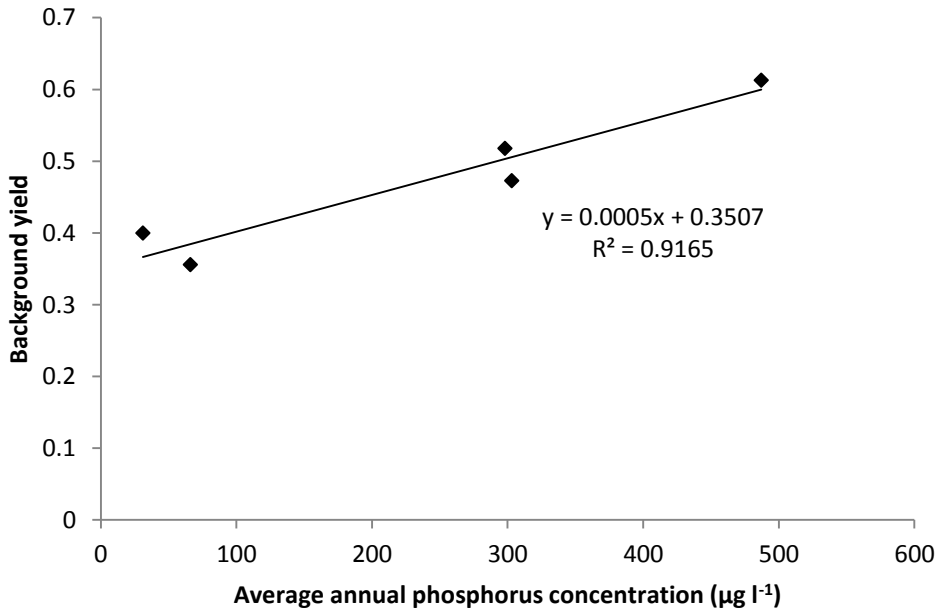


Figure 7.9: Relationship between fluorescence yield and annual average total phosphorus concentration for rivers in the Thames catchment that are a similar length (30 ± 3.5 km).

7.4 Laboratory studies to determine the effect of temperature

Water temperature plays an important role in regulating freshwater ecosystems (Lenane, 2012) and has previously been implicated as being one of the factors responsible for between-year variation in chlorophyll-*a* concentration in the upper Thames catchment (Waylett *et al.*, 2013) and the most important factor in the River Nene, Northamptonshire (Balbi, 2000). It was, therefore, expected that phytoplankton communities would be stressed and have lower fluorescence yields at temperature extremes (both high and low). The average temperature across the 22 sites in the Thames catchment during the 15 week monitoring period was relatively stable, ranging from 12.7 to 15.7 °C. A regression model of temperature against background yield found the relationship between the two variables to be significant ($p = 0.005$). Consequently, further laboratory experiments were undertaken to examine the effects of temperature on fluorescence yield in greater detail. The methodology for these is given earlier in this chapter (Section 7.2.1).

7.4.1 Results and discussion

The resulting water temperatures in each controlled temperature room were close to the room set point with minimal temperature variation between samples. In the 10 °C room, water temperature ranged from 9.5 to 10.6 °C (mean = 10.06 °C). In the 15 °C room, temperature ranged from 13.8 to 14.6 °C (mean = 14.15 °C) and in the 25 °C room, it ranged from 23.3 to 23.9 °C (mean = 23.61 °C). The addition of iron chloride solution successfully reduced SRP concentration of the river water from *ca.* 330 µg l^{-1} to between 17 and 165 µg l^{-1} (Figure 7.10). As the water for the experiment was all collected from the same site (the River Thames at Wallingford), there was no confounding effect of river length on fluorescence yield.

Phosphorus concentration was found to have no significant effect on fluorescence yield in the 15 and 25 °C treatments ($p = 0.348$ and 0.439 respectively). There was, however, a significant relationship ($p = 0.002$) between yield and phosphorus concentration in the lowest temperature treatment (10 °C) (Figure 7.10). This suggests a lower resilience of PS II at lower temperatures and can be related to enzyme activity (which controls the photosynthesis / fluorescence process) being

reduced at lower temperatures. The regression equation for this relationship is given in Equation 6.2.

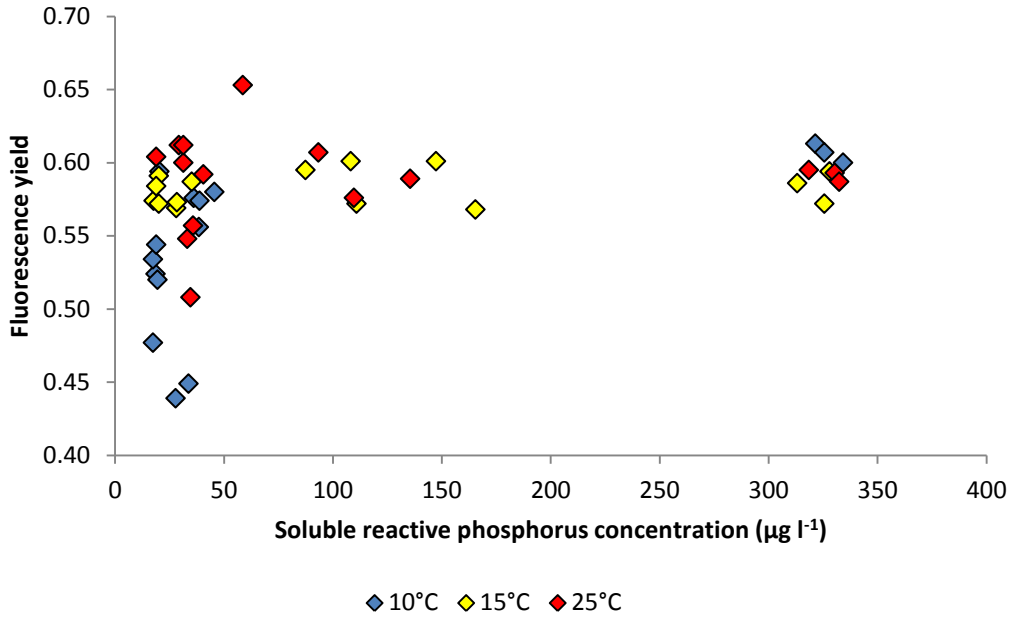


Figure 7.10: The relationship between fluorescence yield and soluble reactive phosphorus concentration at 10 °C (blue), 15 °C (yellow) and 25 °C (red) temperatures.

$$\text{Yield\_10 °C} = 0.523 + 0.000260 * \text{SRP\_10 °C}$$

[Equation 7.2]

Prior to the experiment, the fluorescence yield was 0.614. (S.E. = 0.002). At 10 °C, phytoplankton communities were not stressed at ambient phosphorus concentrations (337 µg l^{-1}) with an average yield from three replicates of 0.607 (S.E. = 0.004). Reducing SRP concentration to *ca.* 30 µg l^{-1} by the addition of iron chloride caused a decrease in yield to *ca.* 0.450 in some of the replicate samples (Figure 7.10). The size of the decrease in yield was not as large in all replicates suggesting phytoplankton communities may have been utilising cellular phosphorus stores to maintain fluorescence yield. At 25 °C, there was also a lower resilience of PS II at lower nutrient concentrations with yields decreasing from 0.590 to 0.500 as SRP was

decreased from 327 µg l^{-1} to *ca.* 30 µg l^{-1} although this was not significant (Figure 7.10).

Replicates with the largest iron addition (at all temperatures) were re-spiked with a phosphorus solution the following morning and yield re-measured after allowing two hours for recovery. This confirmed that the observed decrease in yield was as a result of temperature and nutrient stress and not the effect of the iron addition somehow inhibiting the phytoplankton communities. The resulting SRP concentrations in the treated water were comparable to that of the untreated river water (Table 7.2). At all temperatures, there was no difference in yield between treated and untreated water, once SRP concentrations were similar. Yields recorded were between 0.574 and 0.629 suggesting phytoplankton communities were not stressed. In the 10 °C treatment, where yield was affected by lower phosphorus concentrations, PS II made a full recovery (once phosphorus was re-added) and phytoplankton communities exhibited less stress with yield recovering to an average of 0.610 (S.E. = 0.009) (compared to average yields of 0.605 (S.E.= 0.007) in the untreated river water).

Table 7.2: Soluble reactive phosphorus concentrations (µg l^{-1}) recorded in three replicates of river water and river water dosed with iron and phosphorus at each temperature.

	10 °C		15 °C		25 °C	
	Iron treatment	River water	Iron treatment	River water	Iron treatment	River water
A	272.1	324.9	392.6	296.8	421.6	347.8
B	239.8	306.2	410.1	310.4	346.9	343.9
C	364.4	314.3	299.1	320.2	387.5	346.6

7.5 Conclusions

This chapter set out to examine spatial and temporal patterns in fluorescence yield across the Thames catchment. Changes in yield were related to changes in physical and chemical determinants at 22 sites across the Thames catchment. This allowed patterns to be quantified and the annual collapse of the phytoplankton bloom was analysed in terms of phytoplankton stress response.

As in previous years, a phytoplankton bloom occurred in 2013, peaking at most sites on 13th May 2013. Through the bloom, yield decreased at nine of the sites across the Thames catchment. These sites were generally located in the lower part of the catchment. River length (a proxy for residence time) was found to be a dominant factor in controlling fluorescence yield and stress level. Shorter rivers generally had lower yields, indicating phytoplankton communities were more stressed than in longer rivers. Phosphorus concentration is often cited as the dominant factor in controlling algal blooms. However, this study found that phosphorus did not have any effect on yield until the confounding influence of length was removed. Phosphorus was also determined to be an important factor in controlling yield at lower temperatures. In a similar way to the benthic community, it was found that phytoplankton communities could adapt to changes in phosphorus concentrations. This was illustrated in the communities in the River Kennet which had yields close to the optimum (of 0.65) despite SRP concentrations being below 100 µg l^{-1} following a series of major STW improvements over the last decade.

The present study is the first time fast repetition rate fluorescence has been used to examine yields in a large-scale, long duration freshwater study (22 individual sites covering 17 rivers over 15 weeks). The study has provided useful insights into the patterns and trends in yield across the Thames catchment though as the best subsets regression showed, yield is influenced by a multitude of factors. Maintaining fluorescence yield is a fine balance of all of these factors, specifically residence time, phosphorus availability, self-shading and community composition. It has not been possible, therefore, to determine a single factor that can explain the annual collapse of the phytoplankton bloom.

A problem with interpreting the effect these factors have on fluorescence yield is that most of these physical and chemical conditions tend to co-correlate with each other in river catchments. For instance, large rivers in the UK will tend to have large phytoplankton biomass, long residence time (often further increased by the presence of weirs and connection to canals), and also tend to have the highest nutrient concentrations (due to proximity of major towns to large rivers). This makes it particularly difficult to disentangle the effect of one parameter from another. It is because of this that there is a need to undertake laboratory studies, where it is

possible to manipulate one variable at a time and examine the response in fluorescence yield.

The laboratory study conducted showed phytoplankton communities were more stressed at lower phosphorus concentrations at lower temperatures. Resupplying phosphorus resulted in a recovery in fluorescence yield demonstrating that iron addition was not inhibiting periphyton communities. Chapter Three of this thesis showed the importance of shading in controlling periphyton biomass and quality as a food resource. Future laboratory fluorescence yield studies should examine whether or not shading would induce stress in phytoplankton communities (and lower fluorescence yield). To further understand what is happening, future field studies need to be undertaken at a much higher temporal resolution (than weekly). Previous work examining nutrient dynamics has illustrated the loss of process understanding and ability of data to provide useful insights at low (weekly) resolution (Bowes *et al.*, 2009b). Future work would also need to consider the simultaneous use of a red PhytoFlash which is specifically optimised to examine the responses of the cyanobacteria functional group within the phytoplankton community.

Chapter 8: Conclusion

This thesis has used novel portable in-stream flume mesocosms, developing the pilot study of Bowes *et al.* (2007), to quantify a phosphorus-limiting threshold for periphyton growth in a range of UK rivers. This is the phosphorus concentration at which adding phosphorus results in no further change to periphyton biomass accrual, but reducing phosphorus concentration results in a reduced periphyton biomass accrual rate. The flume mesocosms allowed phosphorus concentration to be simultaneously increased and reduced in a controlled way, while removing variability and interactions with other factors known to affect periphyton biomass accrual. This thesis has also investigated the importance of light intensity in controlling periphyton biomass accrual and examined factors controlling phytoplankton blooms and community stress. These studies have allowed conclusions to be drawn concerning catchment management and provide important information about the best way for the UK to meet the requirements of the Water Framework Directive. The main conclusions of this thesis (in the context of the research questions posed in Section 1.11) are summarised below.

8.1 What is the phosphorus-limiting threshold in UK rivers?

The flume methodology has now been applied to five rivers with ambient SRP concentrations ranging from 15 to 225 µg l^{-1}. In moderately phosphorus enriched rivers (SRP concentration of 40 to 100 µg l^{-1}), the ambient phosphorus concentration has always been at the phosphorus-limiting threshold. In the heavily enriched river (River Thames, SRP concentration = 225 µg l^{-1}), the phosphorus-limiting threshold was quantified as approximately 100 µg l^{-1} (Bowes *et al.*, 2012a). This confirms that there is not a UK-wide phosphorus-limiting threshold that can be applied to all UK rivers, or even particular river typologies, as all rivers investigated within this thesis and previous studies have produced different values for the phosphorus-limiting threshold.

The phosphorus-limiting threshold was determined using traditional quantitative techniques of estimating periphyton biomass; chlorophyll-*a* concentration, ash free

dry mass and autotrophic index. New qualitative techniques such as flow cytometry, alongside more traditional qualitative techniques such as algal identification by microscopy, provide greater insights into changes in biofilm community structure with changing nutrient concentrations. It is important that, in the future, quantitative and qualitative techniques are used simultaneously to provide an overview of the effects of changing nutrient concentration in UK rivers. As the research in the preceding chapters has shown, although the phosphorus-limiting threshold was always the ambient phosphorus concentration, there was a lower ecological phosphorus threshold across all rivers of *ca.* 30 µg l^{-1}. The lower threshold was only evident when examining data from qualitative techniques (flow cytometry and diatom identification / TDI calculation).

The experiments on the River Lambourn and River Frome decreased SRP concentration to *ca.* 30 µg l^{-1}, while the experiment on the River Rede increased SRP concentration to *ca.* 30 µg l^{-1}. In all three rivers, TDI score was lower below 30 µg l^{-1} (indicating a better ecological status) with diatom communities being dominated by more nutrient sensitive species. In the case of the River Frome, the change in TDI score at SRP concentrations of 30 µg l^{-1} was sufficient to change the ecological status (based on diatom communities) from moderate to high. Where flow cytometry analysis was conducted in the River Lambourn, there were significant changes in the proportions of different functional groups within the periphyton biofilm. Below SRP concentrations of 30 µg l^{-1}, cyanobacteria comprised *ca.* 10 % of the autotrophic functional groups within the periphyton community. At ambient SRP concentrations (45 µg l^{-1}), cyanobacteria had a much greater dominance within the biofilm, comprising *ca.* 50 % of the autotrophic functional groups. This thesis has shown that this ecological phosphorus threshold may provide a target phosphorus concentration for all UK rivers, which will result in the good ecological status needed to comply with the WFD.

8.1.1 Is phosphorus the limiting nutrient in UK rivers?

The results from this thesis, Bowes *et al.* (2010a) and Bowes *et al.* (2012a) challenge the traditional belief that phosphorus is the limiting nutrient in UK rivers. The five flume experiments have shown that phosphorus on its own was never limiting

periphyton biomass accrual. Even in the River Rede, where ambient SRP concentrations were *ca.* 15 µg l^{-1}, a sustained nine-fold increase in SRP concentration for the duration of the nine day experiment had no effect on periphyton biomass accrual rate. It was only when phosphorus and nitrogen were added simultaneously that a biomass response was evident, suggesting periphyton communities in the River Rede were sequentially co-limited by phosphorus and nitrogen. Phosphorus and nitrogen were in excess of periphyton growth requirements in all other study rivers. This observation regarding nutrient limitation has important implications for UK policy relating to water quality and pollution mitigation which is primarily focussed on the reduction of overall phosphorus concentrations and avoiding phosphorus spikes from intermittent pollution incidents.

8.1.2 Are periphyton communities able to adapt to reduced phosphorus concentrations?

The flume experiments on the River Frome (Chapter Five) and those of Bowes *et al.* (2007) using streamside flumes at the same location, have shown for the first time that periphyton communities adapt to lower phosphorus concentrations. In both studies, maximum biomass accrual occurred at the ambient concentration and increasing SRP concentration had no significant effect. This was despite mean ambient SRP concentration being reduced from 90 to 65 µg l^{-1} between these two studies. The Bowes *et al.* (2007) study showed that in 2005, such a reduction in phosphorus concentration (from 90 to 65 µg l^{-1}) would have reduced periphyton accrual rate by *ca.* 30 %, and phosphorus would be truly limiting (i.e. on the positive gradient of the limitation curve (Figure 5.3). The repeat experiment in 2012 (Chapter Five) showed that periphyton were again growing at their maximum rate, and the phosphorus-limiting threshold had shifted to the new ambient river concentration (Figure 5.12).

The 2012 study demonstrated that further reducing the SRP concentration in the River Frome to 46 µg l^{-1} resulted in a 20 % decrease in chlorophyll-*a* concentration. However, TDI scores did not change until SRP concentrations below 30 µg l^{-1} were achieved (the suggested ecological phosphorus threshold). From this thesis, it is proposed that at concentrations greater than the ecological phosphorus threshold,

reductions in phosphorus concentration can be viewed as a temporary perturbation to the system. Periphyton community structure has not undergone a major change, and so communities are able to recover and ultimately accrue maximum biomass again at the lowered ambient phosphorus concentration. It is only when there is a large shift in community structure and SRP concentrations are reduced below the ecological phosphorus threshold that changes in periphyton biomass accrual are likely to become permanent and communities are no longer able to adapt. This might reflect the fact that when SRP concentrations are reduced to < 30 µg l^{-1}, periphyton communities become 'truly phosphorus limited'. Periphyton communities being truly limited at SRP concentrations < 30 µg l^{-1} is further supported by data from the River Rede experiment. The River Rede was the only study river that had an ambient SRP concentration of below 30 µg l^{-1}, and also the only river that exhibited nutrient limitation. Therefore, the ultimate target concentration for SRP concentration in UK rivers should be the ecological phosphorus threshold of 30 µg l^{-1}. Defining the target phosphorus concentration as 30 µg l^{-1} is also supported by findings from recently published studies in other countries including the USA (Suplee *et al.*, 2012) and Canada (Chambers *et al.*, 2012).

8.2 Can other abiotic variables be manipulated to reduce periphyton biomass and improve ecological status?

In addition to nutrient concentration, the experiments on the River Lambourn (Chapter Three) manipulated light intensity. At ambient and enriched nutrient concentrations, this resulted in an approximate 40 % reduction in periphyton biomass accrual in the shaded treatment, suggesting it is possible to manipulate other factors known to affect periphyton accrual and improve ecological status. Furthermore, analysis of elemental stoichiometry showed shaded treatments to have lower carbon: phosphorus ratios compared to unshaded treatments at the same nutrient concentration. A lower carbon: phosphorus ratio is indicative of periphyton that is of higher quality (greater nutritional value) to grazing invertebrates. Therefore, shading resulted in less periphyton (which benefits macrophyte species) that is of a higher quality (which benefits invertebrate species).

8.3 Are limiting phosphorus concentrations the cause of stress and the collapse of annual phytoplankton blooms?

Chapter Seven used a PhytoFlash fast repetition rate fluorometer to examine patterns and trends in fluorescence yield across the Thames catchment to try to identify the cause of the annual phytoplankton bloom and its subsequent collapse. Due to the complex, interrelated nature of variables controlling phytoplankton growth, no single factor could be determined as being the cause of phytoplankton community stress / the collapse of the chlorophyll bloom. This underlines the importance of taking a holistic view to water quality management.

Residence time (river length) was found to be an important variable in controlling phytoplankton growth and masked the phosphorus response. When rivers of a similar length were examined, phosphorus concentration was found to have a significant effect in controlling photosynthetic yield. Rivers with lower phosphorus concentrations exhibited lower fluorescence yields indicating phytoplankton communities were more stressed than when phosphorus concentrations were higher. Furthermore, in the rivers with the largest blooms (the lower River Thames at Sonning, Runnymede and the Jubilee River), yields dramatically decreased when SRP concentrations fell below 30 µg l^{-1}. Phytoplankton communities were able to maintain fluorescence yields for approximately one week after SRP depletion suggesting communities were utilising internal phosphorus stores. Once these were depleted, communities became stressed and fluorescence yields only recovered after the collapse of the bloom resulted in an increased SRP concentration. The fact that communities became stressed at the lowest SRP concentrations suggests that the ecological phosphorus threshold derived by the flume experiments for periphyton communities (30 µg l^{-1}) holds true in phytoplankton communities.

8.4 Recommendations for future research

While answering specific key questions, this research has raised a number of additional questions which need to be addressed through further research. The studies within this thesis focussed primarily on the effects of phosphorus concentration. While some attention was paid to the role of nitrogen in regulating periphyton biomass and community structure, the impacts of other macronutrients,

specifically carbon and silicon were ignored. Future experiments should therefore incorporate silicon and carbon treatments. Silicon, in particular, has been shown to regulate diatom communities and could be limiting in some rivers.

All flume experiments were run until maximum periphyton biomass had accrued and sloughing appeared imminent (6 to 11 days). Compared to other nutrient-limitation / periphyton community studies, this was a relatively short timescale. A future experiment should run for comparable timescales (4 – 6 weeks) in order to directly compare chlorophyll-*a* concentrations and AFDM with these 'traditional' field techniques. This would also allow community succession to be investigated through the use of flow cytometry. This was not done in the current research due to the significant resources that would be required to run the flume experiments for this extended time period.

The experiments in the River Lambourn (Chapter Three) illustrated the importance of regulating light intensity to control periphyton biomass. As stated above, this could have important policy implications in meeting the requirements of the WFD. Currently, the Environment Agency is planting riparian shading across many catchments with the aim of reducing stream temperatures, as part of their "Keeping Rivers Cool" project (Lenane, 2012). To test whether the findings from the River Lambourn experiment apply on a catchment scale, it would be possible to complete a before-after-control-impact (BACI) experiment alongside these Environment Agency activities. This would determine the true effect shading has on periphyton biomass and inform water quality policy in the future.

The River Rede study (Chapter Four) indicated the presence of co-limitation of periphyton communities by phosphorus and nitrogen. Further work is needed to examine this, specifically, adding nitrogen along the entire phosphorus concentration range. Adding nitrogen at ambient SRP concentrations resulted in slightly increased chlorophyll-*a* concentrations and AFDM and a corresponding decrease in internal / stored phosphorus concentration. Adding nitrogen across all phosphorus concentrations would allow a co-limitation threshold to be determined. Another development of the work would be to add phosphorus at a range of concentrations for nine days, and then replace phosphorus with nitrogen addition. Recent work has begun to examine the effects of nutrient enrichment upon enzyme activity in the

periphyton microbial community (Lang *et al.*, 2012). This should be looked at in the context of the flume experiments, specifically quantifying alkaline phosphatase and nitrogenase activity to more accurately determine nutrient-limiting thresholds.

The work on the River Frome led to defining an ecological phosphorus threshold (30 µg l^{-1}). If phosphorus concentrations are lowered, but remain greater than this threshold, it seems that periphyton communities adapt and ultimately produce maximum biomass at the lowered phosphorus concentration (as seen in Chapter Five). A key question left unanswered by the current research is 'how long does it take periphyton communities to adapt to lowered phosphorus concentrations?' Future work should be undertaken to examine these questions of recovery time.

A moderately-enriched river (40 – 100 µg SRP l^{-1}) that is about to have ambient phosphorus concentrations reduced by the installation of STW phosphorus-stripping could be used as a site for a future flume experiment. A standard flume experiment with phosphorus addition and reduction treatments would need to be run prior to any alteration in river phosphorus concentration. This would define the current phosphorus-limiting threshold, which, based on the experiments presented, is likely to be the ambient concentration. The flume experiment would then need to be repeated at regular intervals following this reduction in river phosphorus concentration, to determine how long it takes for the periphyton community to recover from the perturbation and to re-adjust its phosphorus-limiting threshold to the new ambient concentration.

For the first time, this thesis has presented fluorescence yield data collected in a large-scale freshwater study. This has provided useful insights into stress and behaviour of phytoplankton communities. This initial study has shown that FrrF data needs to be collected at higher (sub-daily) frequency to gain real insights into the controls on algal blooms and phytoplankton dynamics. Therefore, future work using the PhytoFlash, should consider yield measurements being made at hourly intervals, by *in-situ* deployment. This would need to be done alongside high resolution physical and chemical water quality data to allow accurate interpretation and analysis.

8.5 Concluding remarks

This thesis has challenged traditional beliefs that phosphorus is the limiting nutrient in freshwater ecosystems and has provided insights on how to best meet the requirements of the WFD and improve ecological status. It provides explanation as to why many phosphorus mitigation schemes are not resulting in the expected ecological improvements. In many UK rivers, further phosphorus reductions will be difficult and expensive to achieve. Meeting the proposed ecological phosphorus threshold of 30 µg l^{-1} is unrealistic for many rivers in the agriculturally intensive, heavily populated environment that is the UK. Resources may need to be refocused on improving smaller, rural STW in cleaner, headwater streams, rather than large STW discharging into nutrient-enriched rivers, to achieve this 30 µg l^{-1} SRP target that should finally deliver permanent ecological improvements. For lowland, more urban rivers (as the work on the River Lambourn has shown) the simple introduction of riparian shading is an effective way of reducing periphyton biomass accrual and improving food resource quality. This has positive ecological effects and will assist the UK in meeting the WFD requirement of 'good' ecological and chemical status.

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Appendices

App**endix A: Effectiveness of different chemical forms of iron in reducing soluble reactive phosphorus concentration.**

To determine the most effective chemical form of iron in reducing soluble reactive phosphorus (SRP) concentration, a bulk (4 l) river water sample was collected from the main channel of the River Lambourn on 4th May 2012. This was returned to the laboratory within one hour, where 400 ml of the unfiltered river water was placed in to four individual beakers. The SRP concentration in each beaker was determined (according to the methods presented in Section 2.4.2) and 0.2 ml of a different form of iron solution was added to three of the four beakers. The fourth beaker had nothing added to it so acted as a control.

The iron solutions were made up by dissolving 3 g of compound in 200 ml of deionised water (so that they were the same concentration as the stock solutions being added to the flumes). The different forms of iron tested were iron (II) sulphate (crystal), iron (II) sulphate (powder) and iron (III) chloride. The SRP concentration of each beaker was re-measured after two and five minutes (Table A.1). Based on this it was decided that iron (III) chloride was the most effective form of iron in reducing SRP concentration so was the compound of choice for future experiments.

Table A.1: Changes in soluble reactive phosphorus concentration (µg l^{-1}) of River Lambourn sub-samples after iron had been added.

	Time (minutes)		
Form of iron	0	2	5
Iron (II) sulphate (crystal)	46.2	36.8	24.0
Iron (II) sulphate (powder)	46.5	39.6	29.6
Iron (III) chloride	44.9	22.0	21.9
Control	45.3	45.6	45.2

Appendix B: List and counts of diatom species and sensitivity values identified from samples collected in the River Lambourn, West Berkshire and trophic diatom index calculations.

Downstream

Flume 1 – P addition – average SRP = 161.7 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	3	1	4	12
Achnanthes	*oblongella*	4	1	2	8
Achnanthidium	*biasolettiana*	8	3	4	32
Achnanthidium	*minutissimum*	11	4	2	22
Amphora	*libyca*	4	1	4	16
Campylodiscus		3	1		
Cocconeis	*pediculus*	34	11	4	136
Cocconeis	*placentula*	10	3	3	30
Cyclotella	*meneghiniana*	25	8	4	100
Cymbella	*affinis*	6	2	1	6
Cymbella	*lanceolata*	6	2	2	12
Diatoma	*vulgare*	8	3	5	40
Fragilaria	*bidens*	3	1	3	9
Fragilaria	*capucina*	16	5	1	16
Fragilaria	*capucina var. rumpens*	5	2	2	10
Fragilaria	*vaucheriae*	4	1	4	16
Fragilariforma	*virescens*	3	1	3	9
Gomphonema	*truncatum*	6	2	3	18
Navicula	*lanceolata*	14	5	4	56
Navicula	*menisculus*	4	1	5	20
Navicula	*tripuncta*	9	3	4	36
Nitzschia	*amphibia*	11	4	5	55
Nitzschia	*filiformis*	7	2	4	28
Nitzschia	*fonticola*	6	2	4	24
Nitzschia	*linearis*	16	5	4	64
Nitzschia	*recta*	11	4	4	44
Planothidium		8	3	2	16
Pseudostaurosira	*brevistriata*	8	3	4	32
Rhoicosphenia	*abbreviata*	6	2	4	24
Surirella	*angusta*	4	1	3	12
Synedra	*acus*	5	2	3	15
Synedra	*rumpens*	5	2	2	10
Synedra	*ulna*	27	9	2	54
Sum		300			982
Weighted mean sensitivity		3.27			
Trophic diatom index		57			

Flume 2 – Control – average SRP = 54.2 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes		10	3	3	30
Achnanthidium	*minutissimum*	3	1	2	6
Amphora		5	2	4	20
Cocconeis	*placentula*	35	12	2	70
Cyclotella	*meneghiniana*	11	4	4	44
Cymbella	*affinis*	9	3	1	9
Diatoma	*vulgare*	4	1	5	20
Fragilaria		10	3	2	20
Fragilaria	*bidens*	7	2	3	21
Fragilaria	*capucina*	32	11	1	32
Gomphonema	*minutum*	4	1	4	16
Gomphonema	*olivaceum*	4	1	3	12
Gomphonema	*truncatum*	4	1	3	12
Navicula	*lanceolata*	9	3	4	36
Navicula	*tripuncta*	4	1	4	16
Nitzschia	*amphibia*	21	7	5	105
Nitzschia	*linearis*	14	5	4	56
Nitzschia	*palea*	11	4	4	44
Nitzschia	*recta*	5	2	4	20
Nitzschia	*sigmoidea*	4	1	3	12
Pinnularia		1	< 1	2	2
Planothidium		13	4	2	26
Reimeria	*sinuata*	7	2	3	21
Staurosirella	*pinnata*	7	2	4	28
Synedra	*acus*	6	2	3	18
Synedra	*parasitica*	4	1	5	20
Synedra	*ulna*	56	19	2	112
Sum		300			828
Weighted mean sensitivity		2.76			
Trophic diatom index		44			

Flume 6 – Fe addition – average SRP = 23.6 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	3	1	4	12
Achnanthes	*oblongella*	10	3	2	20
Achnanthidium	*biasolettiana*	4	1	4	16
Cocconeis	*pediculus*	17	6	4	68
Cocconeis	*placentula*	16	5	3	48
Cyclotella	*meneghiniana*	7	2	4	28
Cymbella	*affinis*	6	2	1	6
Diatoma	*vulgare*	7	2	5	35
Eunotia	*exigua*	10	3	1	10
Fragilaria	*capucina*	46	15	1	46
Gomphonema	*olivaceoides*	31	10	2	62
Navicula	*cari*	5	2	4	20
Navicula	*cryptonella*	9	3	5	45
Navicula	*lanceolata*	16	5	4	64
Nitzschia	*amphibia*	4	1	5	20
Nitzschia	*capitellata*	3	1	5	15
Nitzschia	*inconspicua*	3	1	5	15
Nitzschia	*linearis*	7	2	4	28
Nitzschia	*sublinearis*	3	1	2	6
Planothidium		7	2	2	14
Rhoicosphenia	*abbreviata*	5	2	4	20
Reimeria	*sinuata*	6	2	4	24
Synedra	*rumpens*	4	1	2	8
Synedra	*ulna*	71	24	2	142
Sum		300			772
Weighted mean sensitivity		2.57			
Trophic diatom index		39			

Upstream

Flume 2 – PN addition – average SRP = 122.1 µg l^{-1}, average NO_3 – N = 7.92 mg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	5	2	4	20
Amphipleura	*pellucida*	1	< 1	1	1
Amphora	*pediculus*	4	1	4	16
Cocconeis	*pediculus*	30	10	4	120
Cocconeis	*placentula*	12	4	2	24
Cyclotella	*meneghiniana*	29	10	4	116
Cymbella	*affinis*	4	1	1	4
Cymbella	*cistula*	7	2	2	14
Diatoma	*vulgare*	6	2	5	30
Encyonema	*silesiacum*	3	1	3	9
Fragilaria		12	4	4	48
Fragilaria	*capucina*	20	7	1	20
Fragilaria	*exigua*	6	2	4	24
Gomphonema	*olivaceum*	6	2	3	18
Meridion	*circulare*	3	1	1	3
Navicula	*angustata*	5	2	5	25
Navicula	*cryptocephala*	6	2	4	24
Navicula	*lanceolata*	6	2	4	24
Navicula	*menisculus*	6	2	5	30
Nitzschia	*disputata*	6	2	3	18
Nitzschia	*filiformis*	4	1	4	16
Nitzschia	*fonticola*	9	3	4	36
Nitzschia	*linearis*	26	9	4	104
Nitzschia	*recta*	11	4	4	44
Nitzschia	*sigmoidea*	6	2	3	18
Planothidium	*lanceolata*	7	2	4	28
Reimeria	*sinuate*	5	2	3	15
Staurosirella	*elliptica*	4	1	4	16
Synedra	*acus*	14	5	3	42
Synedra	*ulna*	37	12	2	74
Sum		300			981
Weighted mean sensitivity		3.27			
Trophic diatom index		57			

Flume 3 – Control – Average SRP = 42.8 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	4	1	2	8
Achnanthes	*oblongella*	4	1	2	8
Amphipleura	*pellucida*	2	1	1	2
Cocconeis	*pediculus*	31	10	4	124
Cocconeis	*placentula*	15	5	2	30
Cyclotella	*meneghiniana*	19	6	4	76
Cymatopleura	*librile*	1	< 1	4	4
Cymbella		4	1	2	8
Cymbella	*affinis*	2	1	1	2
Diatoma	*vulgare*	5	2	5	25
Ellerbeckia	*arenaria*	2	1	5	10
Fragilaria		3	1	4	12
Fragilaria	*capucina*	49	16	1	49
Gomphonema	*olivaceum*	7	2	3	21
Gomphonema	*truncatum*	4	1	4	16
Navicula	*gregaria*	2	1	3	6
Navicula	*lanceolata*	8	3	4	32
Navicula	*protracta*	4	1	4	16
Navicula	*tripuncta*	8	3	4	32
Nitzschia	*affinis*	5	2	4	20
Nitzschia	*amphibia*	5	2	5	25
Nitzschia	*fonticola*	6	2	4	24
Nitzschia	*linearis*	21	7	4	84
Nitzschia	*recta*	4	1	4	16
Planothidium	*lanceolata*	6	2	4	24
Psammothidium	*subatomoides*	3	1	2	6
Staurosirella	*pinnata*	3	1	4	12
Surirella	*brebissonii*	2	1	5	10
Synedra	*acus*	6	2	3	18
Synedra	*ulna*	65	22	2	130
Sum		300			850
Weighted mean sensitivity		2.83			
Trophic diatom index		46			

Flume 7 – P addition – Average SRP = 146.6 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	8	3	4	32
Achnanthes	*conspicua*	8	3	4	32
Achnanthes	*oblongella*	8	3	2	16
Amphora	*Libyca*	4	1	4	16
Amphora	*pediculus*	10	3	4	40
Cocconeis	*pediculus*	34	11	4	136
Cocconeis	*placentula*	7	2	2	14
Cyclotella	*meneghiniana*	28	9	4	112
Cymbella	*affinis*	8	3	1	8
Cymbella	*lanceolata*	11	4	2	22
Diatoma	*vulgare*	11	4	5	55
Encyonema	*minutum*	12	4	4	48
Encyonema	*silesiacum*	8	3	3	24
Fragilaria		2	1	2	4
Fragilaria	*capucina*	20	7	1	20
Fragilariforma	*virescens*	3	1	3	9
Navicula	*lanceolata*	8	3	4	32
Navicula	*menisculus*	5	2	5	25
Navicula	*tripuncta*	8	3		0
Nitzschia	*amphibia*	8	3	5	40
Nitzschia	*angustata*	9	3	4	36
Nitzschia	*capitellata*	4	1	5	20
Nitzschia	*disputata*	5	2	3	15
Nitzschia	*filiformis*	7	2	4	28
Nitzschia	*linearis*	12	4	4	48
Nitzschia	*recta*	13	4	4	52
Nitzschia	*sigmoidea*	3	1	3	9
Psammothidium	*didymium*	3	1	5	15
Stephanodiscus		2	1	5	10
Synedra	*acus*	5	2	3	15
Synedra	*ulna*	26	9	2	52
Sum		300			985
Weighted mean sensitivity		3.28			
Trophic diatom index		57			

Flume 9 – Fe addition – SRP = 22.7 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	3	1	2	6
Achnanthes	*conspicua*	2	1	4	8
Amphora	*pediculus*	3	1	4	12
Asterionella	*formosa*	14	5	3	42
Cocconeis	*pediculus*	17	6	4	68
Cocconeis	*placentula*	8	3	2	16
Cyclotella	*meneghiniana*	3	1	4	12
Cymbella	*cistula*	3	1	2	6
Diatoma	*vulgare*	3	1	5	15
Encyonema	*minutum*	3	1	4	12
Encyonema	*silesiacum*	12	4	3	36
Eunotia	*exigua*	9	3	1	9
Eunotia	*minor*	5	2	1	5
Fragilaria	*capucina*	40	13	1	40
Fragilariforma	*virescens*	2	1	3	6
Gomphonema	*olivaceoides*	30	10	2	60
Gomphonema	*truncatum*	2	1	4	8
Navicula	*cari*	3	1	4	12
Navicula	*lanceolata*	14	5	4	56
Nitzschia	*angustata*	1	< 1	4	4
Nitzschia	*capitellata*	6	2	5	30
Nitzschia	*linearis*	7	2	4	28
Nitzschia	*filiformis*	2	1	4	8
Planothidium	*lanceolata*	8	3	4	32
Reimeria	*sinuata*	3	1	4	12
Staurosira	*elliptica*	4	1	4	16
Stephanodiscus		1	< 1	5	5
Surirella	*angustata*	1	< 1	3	3
Synedra	*rumpens*	10	3	2	20
Synedra	*ulna*	81	27	2	162
Sum		300			749
Weighted mean sensitivity		2.50			
Trophic diatom index		37			

Table C.1: Water quality data from a longitudinal survey conducted at monthly intervals between May 2012 and April 2013. Values presented are means across the sampling period with sample range given in brackets. The data for Boxford STW effluent was a one off sampling occassion on 1st November 2011.

Site	Site location	Soluble reactive P (µg l^{-1})	Total dissolved P (µg l^{-1})	Total P (µg l^{-1})	Ammonium (mg l^{-1})	Nitrate (mg l^{-1})	Dissolved reactive silicon (mg l^{-1})	Sodium (mg l^{-1})	Potassium (mg l^{-1})	Calcium (mg l^{-1})	Magnesium (mg l^{-1})	Boron (µg l^{-1})	Iron (µg l^{-1})	Manganese (µg l^{-1})	Zinc (µg l^{-1})	Copper (µg l^{-1})	Aluminininum (µg l^{-1})
1	Lambourn	53 (18 - 119)	60 (11 - 136)	67 (13 - 165)	0.042 (0.024 - 0.073)	39.5 (35.9 - 41.6)	7.33 (6.61 - 7.97)	8.5 (5.5 - 14.1)	1.3 (0.9 - 2.0)	108.1 (92.6 - 117.7)	1.7 (1.4 - 1.9)	10.6 (7.8 - 12.5)	26.2 (2.0 - 128.6)	2.6 (0.6 - 10.6)	5.1 (3.0 - 12.4)	1.3 (0.4 - 3.7)	14.9 (< LD* - 74.0)
2	Eastbury	53 (28 - 101)	58 (22 - 127)	66 (23 - 132)	0.039 (0.021 - 0.073)	39.0 (35.0 - 38.5)	6.82 (6.18 - 7.47)	8.8 (7.7 - 11.3)	1.3 (0.9 - 1.8)	108.1 (95.3 - 114.3)	1.6 (1.4 - 1.8)	12.3 (9.2 - 14.0)	16.0 (2.5 - 37.2)	1.7 (0.6 - 3.3)	4.5 (2.0 - 9.4)	1.2 (< LD* - 2.9)	10.9 (< LD* - 35.6)
3	Great Shefford	39 (18 - 79)	42 (12 - 85)	55 (16 - 95)	0.040 (0.006 - 0.062)	35.3 (31.5 - 38.5)	7.39 (6.93 - 8.23)	7.6 (5.6 - 9.4)	1.2 (0.9 - 1.6)	105.0 (98.8 - 110.3)	1.6 (1.4 - 1.8)	11.7 (7.9 - 14.1)	20.6 (7.1 - 40.6)	2.5 (1.0 - 4.1)	3.8 (1.8 - 8.4)	0.7 (< LD* - 1.7)	9.0 (< LD* - 27.3)
4	Weston	42 (15 - 73)	47 (29 - 95)	58 (30 - 59)	0.073 (0.008 - 0.125)	36.3 (33.3 - 38.0)	7.30 (6.79 - 8.02)	8.8 (8.2 - 10.3)	1.5 (1.2 - 1.8)	106.0 (98.9 - 112.7)	1.6 (1.5 - 1.7)	12.2 (7.8 - 15.7)	37.6 (17.3 - 65.7)	3.8 (2.2 - 6.5)	4.3 (1.7 - 12.1)	0.7 (< LD* - 3.3)	10.1 (< LD* - 20.3)
5	Upstream Boxford STW	37 (27 - 67)	42 (21 - 76)	51 (20 - 93)	0.039 (0.011 - 0.071)	34.3 (30.9 - 36.5)	7.40 (6.73 - 8.11)	8.5 (7.8 - 10.2)	1.3 (1.1 - 1.6)	107.6 (100.1 - 112.3)	1.6 (1.5 - 1.7)	12.2 (8.1 - 15.6)	33.7 (13.6 - 72.2)	3.4 (0.9 - 6.1)	4.4 (1.2 - 11.2)	0.9 (0.5 - 2.2)	9.7 (< LD* - 25.6)
6	Downstream Boxford STW	64 (19 - 118)	72 (15 - 131)	91 (32 - 141)	0.056 (0.017 - 0.100)	34.3 (34.1 - 37.5)	7.34 (6.70 - 8.09)	9.2 (8.0 - 11.1)	1.4 (1.1 -1.6)	107.9 (99.9 - 113.9)	1.6 (1.5 - 1.7)	12.2 (8.1 - 15.5)	30.7 (12.7 - 65.2)	3.5 (1.6 - 6.3)	5.2 (1.3 - 22.6)	0.9 (< LD* - 1.9)	9.5 (1.1 - 28.1)
7	Shaw	38 (25 - 70)	48 (20 - 89)	59 (21 - 119)	0.042 (0.009 - 0.073)	33.3 (27.1 - 38.6)	7.01 (5.72 - 7.87)	10.0 (8.4 - 12.7)	1.5 (1.2 - 1.8)	109.7 (93.8 - 115.3)	1.7 (1.5 - 1.8)	13.5 (9.6 - 17.7)	36.2 (10.8 - 106.7)	4.1 (2.1 - 8.6)	4.9 (0.9 - 20.0)	1.0 (< LD* - 4.6)	15.7 (2.9 - 66.3)
	Boxford STW effluent	5190	N/A	5940	1.9	76.2	N/A	N/A	N/A	N/A	N/A	59.0	N/A	N/A	N/A	N/A	N/A

Sites 1 and 2 - n = 11

Sites 3 - 7 - n = 12

* < LD = below limit of detection

Table C.2: Flow data for Site 7 on the sampling occassions during 2012

Date	Flow (m^3 s^{-1})
18/05/2012	1.06
11/06/2012	1.83
09/07/2012	1.63
07/08/2012	1.4
17/09/2012	1.24
22/10/2012	1.5
12/11/2012	2.16
11/12/2012	3.08

Appendix D: List and counts of diatom species and sensitivity values identified from samples collected in the River Rede, Northumberland and trophic diatom index calculations.

Flume 1 – P addition – SRP = 58.4 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	2	1	4	8
Achnanthidium	*minutissimum*	19	6	2	38
Amphora	*libyca*	5	2	4	20
Cocconeis	*pediculus*	4	1	4	16
Cocconeis	*placentula*	2	1	3	6
Cocconeis	*placentula var. pseudolineata*	3	1	3	9
Cyclotella	*meneghiniana*	15	5	4	60
Cymbella	*affinis*	1	< 1	1	1
Cymbella	*delicatula*	1	< 1	1	1
Denticula	*tenuis*	2	1	1	2
Diadesmis	*confervacea*	2	1	3	6
Diatoma	*moniliforme*	2	1	1	2
Diatoma	*tenue*	1	< 1	2	2
Diatoma	*vulgare*	1	< 1	4	4
Diploneis	*elliptica*	2	1	3	6
Diploneis	*oblongella*	3	1	3	9
Encyonema	*gracile*	2	1	2	4
Encyonema	*minutum*	7	2	4	28
Encyonema	*silesiacum*	26	8	3	78
Fragilaria	*capucina (vars. capucina & rumpens)*	1	< 1	2	2
Fragilaria	*capucina var. gracilis*	4	1	2	8
Fragilaria	*vaucheriae*	9	3	3	27
Frustulia	*vulgaris*	1	< 1	1	1
Gomphonema	*olivaceoides*	3	1	2	6
Gomphonema	*olivaceum*	8	3	5	40
Gyrosigma	*acuminatum*	1	< 1	4	4
Navicula	*cryptotenella*	2	1	5	10
Navicula	*radiosa*	1	< 1	2	2
Navicula	*trivialis*	1	< 1	3	3
Neidium	*affine*	3	1	1	3
Neidium	*productum*	1	< 1	2	2
Nitzschia	*angustata*	3	1	4	12
Nitzschia	*capitellata*	13	4	5	65
Nitzschia	*denticula*	1	< 1	4	4
Nitzschia	*dissipata*	5	2	5	25
Nitzschia	*fonticola*	5	2	4	20
Nitzschia	*frustulum*	10	3	5	50
Nitzschia	*gracilis*	63	20	3	189
Nitzschia	*pusilla*	1	< 1	4	4
Nitzschia	*linearis*	4	1	3	12

Nitzschia	*palea*	8	3	4	32
Nitzschia	*paleacea*	6	2	4	24
Nitzschia	*pusilla*	13	4	4	52
Planothidium	*delicatulum*	5	2	5	25
Planothidium	*frequentissimum*	1	< 1	5	5
Rhopalodia	*gibba*	5	2	2	10
Sellaphora	*bacillum*	1	< 1	4	4
Sellaphora	*pupula*	5	2	3	15
Staurosira	*construens*	2	1	4	8
Surirella	*brebissonii*	15	5	5	75
Surirella	*crumena*	1	< 1	3	3
Synedra	*parasitica*	1	< 1	3	3
Synedra	*ulna*	4	1	3	12
Tabularia	*fasciculata*	2	1	4	8
Sum		309			1065
Weighted mean sensitivity		3.45			
Trophic diatom index		61			

Flume 2 – Control – SRP = 16.3 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	16	5	4	64
Achnanthidium	*minutissimum*	51	16	2	102
Amphora	*veneta*	1	< 1	5	5
Cocconeis	*pediculus*	13	4	4	52
Cocconeis	*placentula*	1	< 1	3	3
Cyclotella	*meneghiniana*	5	2	4	20
Cymatopleura	*solea*	4	1	4	16
Cymbella	*delicatula*	2	< 1	1	2
Cymbella	*helvetica*	4	1	2	8
Cymbella	*lanceolata*	10	3	2	20
Denticula	*tenuis*	6	2	1	6
Diatoma	*moniliforme*	1	< 1	1	1
Diatoma	*tenue*	2	1	2	4
Diatoma	*vulgare*	10	3	4	40
Encyonema	*minutum*	11	4	4	44
Encyonema	*silesiacum*	4	1	3	12
Fragilaria	*capucina var. gracilis*	32	10	2	64
Fragilaria	*vaucheriae*	6	2	4	24
Gomphonema	*olivaceoides*	2	1	2	4
Gomphonema	*olivaceum*	2	1	5	10
Gomphonema	*truncatum*	6	2	4	24
Navicula	*capitata*	3	1	5	15
Navicula	*radiosa*	4	1	2	8
Navicula	*rhynchocephala*	1	< 1	4	4
Nitzschia	*acicularis*	64	21	3	192
Nitzschia	*dissipata*	5	2	5	25
Nitzschia	*fonticola*	1	< 1	4	4
Nitzschia	*gracilis*	6	2	3	18
Nitzschia	*lacuum*	4	1	3	12
Nitzschia	*palea*	1	< 1	4	4
Nitzschia	*pusilla*	1	< 1	4	4
Sum		313			916
Weighted mean sensitivity		2.93			
Trophic diatom index		48			

Flume 3 – P addition – SRP = 87.4 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*oblongella*	1	< 1	2	2
Achnanthidium	*microcephalum*	1	< 1	2	2
Achnanthidium	*minutissimum*	33	10	2	66
Aneumastus	*tusculus*	1	< 1	1	1
Cocconeis	*placentula*	7	2	3	21
Ctenophora	*pulchella*	1	< 1	3	3
Cyclotella	*meneghiniana*	8	2	4	32
Cymatopleura	*solea*	1	< 1	4	4
Cymbella	*helvetica*	1	< 1	2	2
Cymbella	*lanceolata*	2	1	2	4
Denticula	*tenuis*	3	1	1	3
Diatoma	*tenue*	4	1	2	8
Diatoma	*vulgare*	5	2	4	20
Encyonema	*caespitosum*	2	1	3	6
Encyonema	*minutum*	2	1	3	6
Encyonema	*silesiacum*	10	3	3	30
Fragilaria	*capucina (vars. capucina & rumpens)*	1	< 1	2	2
Fragilaria	*capucina var. gracilis*	6	2	2	12
Fragilaria	*vaucheriae*	24	7	4	96
Fragilaria	*vaucheriae var. capitellata*	3	1	2	6
Gomphonema	*acuminatum*	1	< 1	3	3
Gomphonema	*clevei*	2	1	3	6
Gomphonema	*olivaceum*	4	1	5	20
Gomphonema	*parvulum*	1	< 1	4	4
Gomphonema	*pumilum*	1	< 1	3	3
Gomphonema	*truncatum*	3	1	4	12
Melosira	*varians*	17	5	5	85
Navicula	*cryptotenella*	4	1	5	20
Navicula	*menisculus*	1	< 1	5	5
Navicula	*radiosa*	3	1	2	6
Navicula	*trivialis*	1	< 1	3	3
Nitzschia	*acicularis*	95	29	3	285
Nitzschia	*amphibia*	3	< 1	5	15
Nitzschia	*dissipata*	7	2	5	35
Nitzschia	*fonticola*	7	2	3	21
Nitzschia	*gracilis*	15	5	3	45
Nitzschia	*linearis*	9	3	3	27
Nitzschia	*palea*	8	2	4	32
Nitzschia	*paleacea*	1	< 1	4	4
Nitzschia	*recta*	2	1	3	6
Nitzschia	*sublinearis*	4	1	2	8
Nitzschia	*tubicola*	1	< 1	4	4
Sellaphora	*seminulum*	1	< 1	4	4
Stauroneis	*phoenicenteron*	1	< 1	5	5
Staurosirella	*lapponica*	3	1	4	12
Surirella	*angusta*	6	2	4	24
Surirella	*brebissonii*	8	2	3	24

Synedra	*ulna*	7	2	3	21
Sum		332			1065
Weighted mean sensitivity		3.21			
Trophic diatom index		55			

Flume 5 – P addition – SRP = 38.8 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*minutissimum*	29	9	2	58
Cocconeis	*pediculus*	9	3	4	36
Ctenophora	*pulchella*	4	1	3	12
Cyclotella	*meneghiniana*	12	34	4	48
Cymbella	*helvetica*	2	1	2	4
Cymbella	*lanceolata*	1	< 1	4	4
Denticula	*tenuis*	3	1	1	3
Diatoma	*tenue*	7	2	2	14
Diatoma	*vulgare*	12	4	4	48
Encyonema	*gracile*	1	< 1	2	2
Encyonema	*minutum*	6	2	4	24
Encyonema	*silesiacum*	6	2	3	18
Fragilaria	*capucina var. amphicephala*	1	< 1	5	5
Fragilaria	*capucina (vars. capucina & rumpens)*	19	6	1	19
Fragilaria capucina	*var. gracilis*	10	3	2	20
Fragilaria	*vaucheriae*	4	1	4	16
Gomphonema	*acuminatum*	2	1	3	6
Gomphonema	*clevei*	2	1	3	6
Gomphonema	*gracile*	1	< 1	3	3
Gomphonema	*olivaceoides*	1	< 1	2	2
Gomphonema	*olivaceum*	1	< 1	5	5
Gomphonema	*truncatum*	1	< 1	4	4
Gyrosigma	*acuminatum*	1	< 1	4	4
Melosira	*varians*	10	3	5	50
Meridion	*circulare*	5	2	1	5
Navicula	*cryptotenella*	12	4	5	60
Navicula	*lanceolata*	1	< 1	4	4
Navicula	*minima*	1	< 1	3	3
Navicula	*radiosa*	5	2	2	10
Navicula	*trivialis*	1	< 1	3	3
Nitzschia	*acicularis*	70	21	3	210
Nitzschia	*capitellata*	5	2	5	25
Nitzschia	*dissipata*	10	3	5	50
Nitzschia	*fonticola*	4	1	4	16
Nitzschia	*frustulum*	1	< 1	4	4
Nitzschia	*gracilis*	25	7	3	75
Nitzschia	*linearis*	7	2	3	21
Nitzschia	*palea*	20	6	4	80
Planothidium	*delicatulum*	2	1	5	10
Stephanodiscus		1	< 1	5	5
Surirella	*brebissonii*	9	3	5	45
Surirella	*ovalis*	1	< 1	3	3
Synedra	*ulna*	11	3	3	33
Sum		336			1073
Weighted mean sensitivity		3.19			
Trophic diatom index		55			

Flume 6 – P addition – SRP = 30.1 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	7	2	4	28
Achnanthidium	*minutissimum*	30	10	2	60
Aulacoseira		4	1	2	8
Caloneis	*salina*	1	< 1	2	2
Caloneis	*silicula*	3	1	3	9
Cocconeis	*pediculus*	4	1	4	16
Cocconeis	*placentula*	6	2	3	18
Ctenophora	*pulchella*	3	1	3	9
Cyclotella	*meneghiniana*	5	2	4	20
Cymatopleura	*solea*	2	1	4	8
Cymbella	*helvetica*	2	1	2	4
Cymbella	*lanceolata*	4	1	2	8
Cymbella	*microcephala*	2	1	1	2
Denticula	*tenuis*	2	1	1	2
Diatoma	*tenue*	5	2	2	10
Diatoma	*vulgare*	8	3	4	32
Encyonema	*minutum*	4	1	4	16
Encyonema	*prostratum*	2	1	2	4
Encyonema	*silesiacum*	10	3	3	30
Eunotia	*exigua*	2	1	1	2
Fragilaria	*bidens*	1	< 1	4	4
Fragilaria	*capucina (vars. capucina & rumpens)*	4	1	3	12
Fragilaria	*capucina var. gracilis*	3	1	2	6
Fragilaria	*vaucheriae*	15	5	4	60
Fragilariforma	*virescens*	1	< 1	3	3
Frustulia	*vulgaris*	1	< 1	1	1
Gomphonema	*olivaceum*	1	< 1	5	5
Gomphonema	*pumilum*	1	< 1	3	3
Gomphonema	*truncatum*	3	1	4	12
Melosira	*varians*	2	1	5	10
Navicula	*cryptotenella*	6	2	5	30
Navicula	*molestiformis*	1	< 1	4	4
Navicula	*radiosa*	1	< 1	2	2
Navicula	*rhynchocephala*	2	1	4	8
Nitzschia	*acicularis*	73	24	3	219
Nitzschia	*amphibia*	3	1	5	15
Nitzschia	*capitellata*	2	1	5	10
Nitzschia	*dissipata*	3	1	5	15
Nitzschia	*fonticola*	5	2	4	20
Nitzschia	*frustulum*	4	1	5	20
Nitzschia	*gracilis*	9	3	3	27
Nitzschia	*heufleriana*	2	1	2	4
Nitzschia	*lacuum*	1	< 1	3	3
Nitzschia	*linearis*	10	3	3	30
Nitzschia	*palea*	4	1	4	16
Nitzschia	*paleacea*	4	1	4	16
Nitzschia	*subacicularis*	2	1	4	8
Planothidium	*delicatulum*	10	3	5	50

Planothidium	*frequentissimum*	1	< 1	5	5
Rhopalodia	*gibba*	1	< 1	2	2
Rossithidium		1	< 1	2	2
Sellaphora	*pupula*	3	1	3	9
Surirella	*brebissonii*	8	3	5	40
Surirella	*roba*	1	< 1	1	1
Synedra	*ulna*	8	3	3	24
Tabularia	*fasciculata*	4	1	4	16
Sum		307			1000
Weighted mean sensitivity		3.26			
Trophic diatom index		56			

Flume 9 – P addition – SRP = 130.1 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*biasolettiana*	8	3	4	32
Achnanthidium	*minutissimum*	20	7	2	40
Cocconeis	*pediculus*	2	1	4	8
Cocconeis	*placentula*	7	2	3	21
Cyclotella	*meneghiniana*	4	1	4	16
Cymatopleura	*solea*	2	1	4	8
Cymbella	*helvetica*	4	1	2	8
Cymbella	*lanceolata*	3	1	2	6
Denticula	*tenuis*	2	1	1	2
Diatoma	*tenue*	1	< 1	2	2
Diatoma	*vulgare*	7	2	4	28
Encyonema	*minutum*	9	3	4	36
Encyonema	*silesiacum*	5	2	3	15
Fragilaria	*capucina (vars. capucina & rumpens)*	7	2	1	7
Fragilaria	*capucina var. gracilis*	7	2	2	14
Fragilaria	*vaucheriae*	31	10	4	124
Gomphonema	*clevei*	4	1	3	12
Gomphonema	*olivaceum*	2	1	5	10
Gomphonema	*parvulum*	2	1	4	8
Melosira	*varians*	15	5	5	75
Meridion	*circulare*	4	1	1	4
Navicula	*decussis*	1	< 1	5	5
Navicula	*gregaria*	1	< 1	5	5
Navicula	*lanceolata*	4	1	4	16
Navicula	*trivialis*	10	3	3	30
Navicula	*veneta*	1	< 1	5	5
Neidium	*affine*	1	< 1	1	1
Nitzschia	*acicularis*	44	14	3	132
Nitzschia	*dissipata*	6	2	5	30
Nitzschia	*fonticola*	16	5	4	64
Nitzschia	*gracilis*	4	1	3	12
Nitzschia	*heufleriana*	2	1	2	4
Nitzschia	*lacuum*	2	1	3	6
Nitzschia	*linearis*	7	2	3	21
Nitzschia	*palea*	15	5	4	60
Planothidium	*delicatulum*	14	5	5	70
Reimeria	*sinuata*	1	< 1	4	4
Sellaphora	*pupula*	1	< 1	3	3
Stephanodiscus		1	< 1	5	5
Surirella	*angusta*	1	< 1	4	4
Surirella	*brebissonii*	17	6	5	85
Surirella	*linearis*	3	1	3	9
Synedra	*ulna*	8	3	3	24
Sum		306			1071
Weighted mean sensitivity		3.50			
Trophic diatom index		63			

Flume 10 – PN addition – SRP = 133.8 µg l^{-1}, average NO_3 – N =1.37 mg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*minutissimum*	24	8	2	48
Cocconeis	*pediculus*	5	2	4	20
Cocconeis	*placentula*	1	< 1	3	3
Ctenophora	*pulchella*	3	1	3	9
Cyclotella	*meneghiniana*	10	3	4	40
Cymatopleura	*solea*	3	1	4	12
Cymbella	*lanceolata*	3	1	2	6
Denticula	*tenuis*	3	1	1	3
Diatoma	*moniliforme*	2	1	1	2
Diatoma	*tenue*	3	1	1	3
Diatoma	*vulgare*	6	2	4	24
Encyonema	*caespitosum*	1	< 1	3	3
Encyonema	*minutum*	13	4	4	52
Encyonema	*silesiacum*	7	2	3	21
Eunotia	*minor*	1	< 1	4	4
Fragilaria	*capucina var. amphicephala*	4	1	1	20
Fragilaria	*capucina (vars. capucina & rumpens)*	14	5	1	14
Fragilaria	*capucina var. gracilis*	11	4	2	22
Fragilaria	*vaucheriae*	16	5	4	64
Gomphonema	*clavatum*	1	< 1	3	3
Gomphonema	*clevei*	2	1	3	6
Gomphonema	*minutum*	1	< 1	3	3
Gomphonema	*olivaceoides*	1	< 1	2	2
Gomphonema	*truncatum*	1	< 1	4	4
Gyrosigma	*acuminatum*	1	< 1	4	4
Melosira	*varians*	8	1	5	40
Navicula	*cincta*	1	< 1	3	3
Navicula	*cryptocephala*	3	1	4	12
Navicula	*lanceolata*	4	1	4	16
Navicula	*protracta*	1	< 1	4	4
Navicula	*radiosa*	2	1	4	8
Navicula	*trivialis*	5	2	3	15
Navicula	*veneta*	5	2	5	25
Nitzschia	*acicularis*	48	15	3	144
Nitzschia	*dissipata*	9	3	5	45
Nitzschia	*fonticola*	7	2	4	28
Nitzschia	*frustulum*	2	1	5	10
Nitzschia	*gracilis*	8	3	3	24
Nitzschia	*lacuum*	4	1	3	12
Nitzschia	*linearis*	11	4	3	33
Nitzschia	*palea*	19	6	4	76
Nitzschia	*perminuta*	1	< 1	3	3
Planothidium	*delicatulum*	2	1	5	10
Rhoicosphenia	*abbreviata*	1	< 1	5	5
Sellaphora	*pupula*	4	1	3	12
Staurosira	*construens*	1	< 1	4	4
Stephanodiscus	*brebissonii*	1	< 1	5	5

Surirella		12	4	5	60
Surirella	*roba*	1	< 1	1	1
Synedra	*ulna*	7	2	2	14
Tabellaria	*flocculosa*	2	1	2	4
Tabularia	*fasciculata*	5	2	4	20
Sum		311			1020
Weighted mean sensitivity		3.28			
Trophic diatom index		57			

Flume 11 – N addition – average NO_3 – N = 1.30 mg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthidium	*minutissimum*	45	14	2	90
Cocconeis	*pediculus*	2	1	4	8
Cocconeis	*placentula*	6	2	3	18
Ctenophora	*pulchella*	7	2	3	21
Cyclotella	*meneghiniana*	5	2	4	20
Cymatopleura	*solea*	1	< 1	4	4
Cymbella	*helvetica*	3	1	2	6
Denticula	*tenuis*	7	2	1	7
Diatoma	*tenue*	5	2	2	10
Diatoma	*vulgare*	4	1	4	16
Encyonema	*minutum*	22	7	4	88
Encyonema	*silesiacum*	10	3	3	30
Fragilaria	*capucina (vars. capucina & rumpens)*	21	7	2	42
Fragilaria	*capucina var. gracilis*	13	4	2	26
Fragilaria	*vaucheriae*	13	4	4	52
Gomphonema	*clavatum*	2	1	3	6
Gomphonema	*clevei*	4	1	3	12
Gomphonema	*gracile*	2	1	2	4
Gomphonema	*olivaceum*	2	1	5	10
Gomphonema	*truncatum*	1	< 1	4	4
Gyrosigma	*acuminatum*	1	< 1	4	4
Melosira	*varians*	4	1	5	20
Navicula	*lanceolata*	2	1	4	8
Navicula	*minuscula*	1	< 1	5	5
Navicula	*radiosa*	2	1	2	4
Navicula	*trivialis*	6	2	3	18
Navicula	*viridula*	1	< 1	4	4
Neidium	*affine*	1	< 1	1	1
Nitzschia	*acicularis*	20	6	3	60
Nitzschia	*dissipata*	13	4	5	65
Nitzschia	*epithemoides*	1	< 1	4	4
Nitzschia	*fonticola*	9	3	4	36
Nitzschia	*gracilis*	4	1	3	12
Nitzschia	*heufleriana*	1	< 1	2	2
Nitzschia	*linearis*	12	4	3	36
Nitzschia	*palea*	19	6	4	76
Planothidium	*delicatulum*	8	3	5	40
Planothidium	*rostratum*	9	3	5	45
Sellaphora	*pupula*	1	< 1	3	3
Staurosira	*construens*	2	1	4	8
Surirella	*brebissonii*	8	3	5	40
Surirella	*linearis*	2	1	3	6
Synedra	*ulna*	12	4	3	36
Tabularia	*fasciculata*	3	1	5	15
Sum		317			1022
Weighted mean sensitivity		3.22			
Trophic diatom index		56			

Appendix E: List and counts of diatom species and sensitivity values identified from samples collected in the River Frome, Dorset and trophic diatom index calculations.

Flume 1 – PN addition – average SRP = 129.8 µg l^{-1}, average NO_3-N = 5.69 mg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Cocconeis	*pediculus*	5	2	4	20
Cyclotella	*meneghiniana*	235	78	4	940
Diatoma	*vulgare*	1	< 1	4	4
Encyonema	*silesiacum*	3	1	3	9
Fragilaria	*capucina*	6	2	1	6
Navicula	*cryptocephala*	8	3	4	32
Navicula	*lanceolata*	4	1	4	16
Nitzschia	*angustatula*	6	2	4	24
Nitzschia	*recta*	20	7	3	60
Sellaphora	*bacillum*	1	< 1	4	4
Staurosirella	*lapponica*	1	< 1	4	4
Surirella	*angusta*	3	1	4	12
Surirella	*brebissonii*	4	1	5	20
Synedra	*ulna*	3	1	2	6
Sum		300			1157
Weighted mean sensitivity		3.86			
Trophic diatom index		71			

Flume 2 – Fe addition – average SRP = 46.2 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Cocconeis	*pediculus*	6	2	4	24
Cyclotella	*meneghiniana*	229	76	4	916
Cymbella	*affinis*	4	1	1	4
Diatoma	*vulgare*	7	2	4	28
Fragilaria	*capucina*	3	1	1	3
Fragilaria	*vaucheriae*	2	1	4	8
Fragilariforma	*virescens*	5	2	3	15
Gomphonema	*truncatum*	6	2	4	24
Navicula	*lanceolata*	5	2	4	20
Navicula	*tripunctata*	8	3	5	40
Nitzschia	*amphibia*	13	4	5	65
Planothidium	*delicatulum*	2	1	5	10
Staurosirella	*lapponica*	8	3	4	32
Tabellaria	*flocculosa*	2	1	2	4
Sum		300			1193
Weighted mean sensitivity		3.977			
Trophic diatom index		74			

Flume 6 – P addition – average SRP = 106.2 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Amphora	*libyca*	3	1	4	12
Cocconeis	*pediculus*	3	1	4	12
Cyclotella	*meneghiniana*	231	77	4	924
Diatoma	*vulgare*	5	2	4	20
Encyonema	*minutum*	2	1	4	8
Fragilaria	*capucina*	9	3	1	9
Navicula	*lanceolata*	1	< 1	4	4
Navicula	*tripunctata*	4	1	5	20
Nitzschia	*capitellata*	10	3	5	50
Nitzschia	*fonticola*	22	7	4	88
Nitzschia	*gracilis*	1	< 1	3	3
Planothidium		2	1	5	10
Surirella	*angusta*	2	1	4	8
Surirella	*brebissonii*	3	1	5	15
Synedra	*ulna*	2	1	2	4
Sum		300			1187
Weighted mean sensitivity		3.96			
Trophic diatom index		74			

Flume 7 – Control – average SRP = 64.9 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	4	1	4	16
Cocconeis	*pediculus*	3	1	4	12
Cyclotella	*meneghiniana*	241	80	4	964
Encyonema	*minutum*	2	1	4	8
Navicula	*lanceolata*	6	2	4	24
Nitzschia	*capitellata*	11	4	5	55
Nitzschia	*fonticola*	19	6	4	76
Nitzschia	*linearis*	2	1	3	6
Surirella	*angusta*	3	1	4	12
Synedra	*ulna*	9	3	2	18
Sum		300			1191
Weighted mean sensitivity		3.97			
Trophic diatom index		74			

Flume 8 – Fe addition – average SRP = 25.9 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*oblongella*	4	1	2	8
Achnanthidium	*biasolettiana*	1	< 1	4	4
Cocconeis	*pediculus*	11	4	4	44
Cocconeis	*placentula*	1	< 1	3	3
Cyclotella	*meneghiniana*	94	31	4	376
Diatoma	*vulgare*	5	2	4	20
Encyonema	*silesiacum*	1	< 1	3	3
Fragilaria	*capucina*	8	3	1	8
Gomphonema	*olivaceoides*	21	7	5	105
Navicula	*capitata*	2	1	5	10
Navicula	*capitatoradiata*	2	1	4	8
Navicula	*lanceolata*	5	2	4	20
Nitzschia	*filiformis*	18	6	4	72
Nitzschia	*pusilla*	2	1	4	8
Nitzschia	*recta*	13	4	3	39
Planothidium	*lanceolatum*	4	1	4	16
Staurosirella	*lapponica*	1	< 1	4	4
Surirella	*brebissonii*	2	1	5	10
Synedra	*ulna*	105	35	2	210
Sum		300			968
Weighted mean sensitivity		3.227			
Trophic diatom index		56			

Flume 9 – P addition – average SRP = 154.6 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Amphora	*libyca*	2	1	4	8
Cocconeis	*pediculus*	7	2	4	28
Cyclotella	*meneghiniana*	200	67	4	800
Diatoma	*vulgare*	3	1	4	12
Encyonema	*silesiacum*	2	1	3	6
Fragilaria	*capucina*	6	2	1	6
Gomphonema	*angustatum*	1	< 1	4	4
Gomphonema	*parvulum*	3	1	4	12
Melosira	*varians*	12	4	5	60
Navicula	*lanceolata*	3	1	4	12
Nitzschia	*acicularis*	15	5	3	45
Nitzschia	*amphibia*	2	1	5	10
Nitzschia	*capitellata*	3	1	5	15
Nitzschia	*dissipata*	3	1	5	15
Nitzschia	*recta*	24	8	3	72
Planothidium	*delicatulum*	4	1	5	20
Staurosira	*elliptica*	2	1	4	8
Surirella	*angusta*	3	1	4	12
Surirella	*brebissonii*	2	1	5	10
Synedra	*ulna*	3	1	2	6
Sum		300			1161
Weighted mean sensitivity		3.87			
Trophic diatom index		72			

Flume 10 – N addition – average NO_3-N = 6.24 mg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	1	< 1	4	4
Achnanthes	*oblongella*	2	1	2	4
Cocconeis	*pediculus*	9	3	4	36
Cyclotella	*meneghiniana*	230	77	4	920
Diatoma	*vulgare*	2	1	4	8
Encyonema	*silesiacum*	2	1	3	6
Fragilaria	*capucina*	6	2	1	6
Gomphonema	*parvulum*	1	< 1	4	4
Melosira	*varians*	12	4	5	60
Navicula	*lanceolata*	2	1	4	8
Nitzschia	*filiformis*	8	3	4	32
Nitzschia	*fonticola*	10	3	4	40
Nitzschia	*linearis*	1	< 1	3	3
Planothidium	*lanceolatum*	4	1	4	16
Surirella	*brebissonii*	1	< 1	5	5
Synedra	*ulna*	9	3	2	18
Sum		300			1170
Weighted mean sensitivity		3.90			
Trophic diatom index		73			

Flume 12 – Fe addition – average SRP = 32.6 µg l^{-1}

Genus	Species	Abundance (A)	Percentage	Sensitivity (S)	A x S
Achnanthes	*conspicua*	3	1	4	12
Achnanthidium	*biasolettiana*	1	< 1	4	4
Cocconeis	*pediculus*	6	2	4	24
Cyclotella	*meneghiniana*	128	43	4	512
Diatoma	*vulgare*	4	1	4	16
Encyonema	*silesiacum*	2	1	3	6
Fragilaria	*capucina*	20	7	1	20
Gomphonema	*angustatum*	2	1	4	8
Melosira	*varians*	17	6	5	85
Navicula	*lanceolata*	2	1	4	8
Nitzschia	*acicularis*	14	5	3	42
Nitzschia	*filiformis*	14	5	4	56
Nitzschia	*lacuum*	3	1	3	9
Nitzschia	*recta*	12	4	3	36
Planothidium	*lanceolatum*	2	1	4	8
Surirella	*angusta*	2	1	4	8
Surirella	*brebissonii*	2	1	5	10
Synedra	*ulna*	66	22	2	132
Sum		300			996
Weighted mean sensitivity		3.32			
Trophic diatom index		58			

Table F.1: Water quality data from a longitudinal survey conducted five times between July 2012 and July 2013. Values presented are means across the sampling period with sample range given in brackets.

Site	Site location	River	Soluble reactive P ($\mu g\ l^{-1}$)	Total dissolved P ($\mu g\ l^{-1}$)	Total P ($\mu g\ l^{-1}$)	Ammonium ($mg\ l^{-1}$)	Nitrate ($mg\ l^{-1}$)	Si ($mg\ l^{-1}$)	DOC ($mg\ l^{-1}$)	pH	Alkalinity ($\mu equiv\ l^{-1}$)	Sodium ($mg\ l^{-1}$)	Potassium ($mg\ l^{-1}$)	Calcium ($mg\ l^{-1}$)	Magnesium ($mg\ l^{-1}$)	Boron ($\mu g\ l^{-1}$)	Iron ($\mu g\ l^{-1}$)	Manganese ($\mu g\ l^{-1}$)	Zinc ($\mu g\ l^{-1}$)	Copper ($\mu g\ l^{-1}$)	Aluminiinium ($\mu g\ l^{-1}$)
1	Sandhills	Wraxall Brook	48 (42 - 55)	52 (42 - 56)	69 (62 - 81)	0.052 (0.034 - 0.075)	17.3 (13.2 - 22.5)	4.25 (3.64 - 4.63)	3.9 (2.0 - 7.7)	8.04 (7.97 - 8.13)	3917 (3347 - 4712)	10.3 (9.7 - 10.8)	1.6 (1.2 - 2.2)	89.6 (73.5 - 105.9)	2.4 (2.6 - 2.7)	14.8 (8.1 - 19.5)	79.2 (29.8 - 173.6)	15.9 (8.7 - 25.4)	2.5 (0.8 - 5.3)	2.4 (< LD* - 5.3)	17.8 (3.5 - 48.6)
2	Chilfrome	Frome	88 (72 - 104)	97 (85 - 117)	120 (95 - 140)	0.061 (0.027 - 0.114)	18.3 (13.8 - 23.3)	4.58 (4.41 - 4.97)	3.9 (2.1 - 6.9)	8.00 (7.94 - 8.06)	4187 (3707 - 4824)	10.6 (9.8 - 11.6)	2.0 (1.6 - 2.4)	97.8 (82.4 - 112.5)	2.7 (2.6 - 2.8)	17.7 (11.0 - 22.0)	69.0 (30.1 - 119.3)	18.4 (10.9 - 27.3)	2.5 (1.5 - 6.0)	0.5 (< LD* - 1.4)	14.0 (3.0 - 26.2)
3	Maiden Newton	Hooke	70 (57 - 84)	72 (61 - 89)	96 (74 - 114)	0.055 (0.024 - 0.080)	22.2 (15.8 - 27.6)	4.70 (4.55 - 4.80)	4.0 (2.2 - 7.1)	8.05 (7.94 - 8.22)	4326 (3854 - 4940)	9.9 (9.5 - 10.2)	1.7 (1.3 - 2.1)	97.1 (84.1 - 109.4)	2.6 (2.5 - 2.8)	13.6 (7.9 - 17.1)	73.0 (22.6 - 148.0)	14.3 (10.5 - 18.7)	1.9 (< LD* - 4.0)	0.6 (< LD* - 1.1)	15.6 (2.4 - 25.7)
4	Frampton	Frome	71 (56 - 81)	76 (48 - 102)	90 (59 - 107)	0.046 (0.019 - 0.068)	23.2 (18.0 - 27.0)	4.21 (3.63 - 4.63)	2.8 (1.6 - 5.5)	7.90 (7.80 - 8.10)	4386 (3939 - 4674)	9.9 (9.6 - 10.1)	1.6 (1.3 - 2.0)	98.4 (88.7 - 107.5)	2.5 (2.4 - 2.5)	14.4 (8.1 - 18.5)	46.1 (12.8 - 95.8)	9.6 (5.7 - 18.7)	2.0 (0.7 - 3.0)	0.7 (< LD* - 1.2)	7.1 (< LD* - 24.7)
5	Grimstone	Sydling Water	47 (35 - 62)	46 (41 - 63)	54 (45 - 64)	0.041 (0.033 - 0.055)	25.8 (24.0 - 27.5)	3.98 (3.55 - 4.30)	1.5 (1.3 - 2.0)	7.98 (7.87 - 8.09)	4451 (4285 - 4667)	8.6 (8.2 - 8.8)	0.9 (0.6 - 1.1)	103.0 (95.3 - 109.9)	2.0 (1.9 - 2.0)	9.4 (5.3 - 11.3)	11.8 (4.1 - 37.6)	2.9 (1.8 - 3.8)	5.5 (2.8 - 9.4)	0.5 (< LD* - 1.3)	6.5 (< LD* - 30.0)
6	Bradford Pevrell	Frome	63 (48 - 78)	67 (53 - 81)	85 (63 - 103)	0.048 (0.031 - 0.064)	24.3 (19.3 - 26.6)	4.00 (3.19 -4.45)	2.7 (1.9 - 4.9)	8.00 (7.93 - 8.18)	4442 (4119 - 4663)	9.8 (9.5 - 10.1)	1.5 (1.1 - 1.9)	100.6 (92.1 - 107.6)	2.3 (2.3 - 2.4)	13.2 (7.4 - 16.8)	29.1 (8.7 - 54.7)	7.0 (4.3 - 11.5)	5.0 (2.8 - 10.8)	1.3 (< LD* - 2.2)	10.0 (< LD* - 27.2)
7	Charminster	Cerne	78 (53 - 120)	79 (54 - 124)	90 (64 - 139)	0.042 (0.025 - 0.054)	25.3 (20.8 -29.1)	3.75 (3.22 - 4.11)	1.9 (1.6 - 2.4)	8.07 (7.79 - 8.25)	4544 (4393 - 4659)	8.9 (8.7 - 9.2)	1.1 (0.9 - 1.3)	103.8 (99.0 - 1-8.7)	2.0 (1.9 - 2.0)	11.6 (6.9 - 14.9)	13.4 (5.6 - 29.2)	3.9 (1.7 - 7.5)	1.8 (0.0 - 2.9)	< LD* (< LD* - 20.1)	6.0 (< LD* - 20.1)
8	Lower Bockhampton (no STW)	Frome	61 (42 - 76)	63 (46 - 79)	92 (61 - 110)	0.046 (0.042 - 0.050)	28.3 (25.3 - 30.5)	3.83 (3.13 - 4.42)	2.6 (1.9 - 4.1)	8.01 (7.93 - 8.14)	4435 (4202 - 4700)	12.4 (10.8 - 14.4)	1.9 (1.6 - 2.3)	102.2 (97.2 - 106.6)	2.4 (2.2 - 2.5)	16.4 (12.7 - 19.0)	17.9 (11.3 - 29.7)	5.3 (4.8 - 5.8)	2.9 (1.9 - 4.5)	0.9 (<LD* - 1.3)	2.8 (< LD* - 7.1)
9	Lower Bockhampton (STW)	Frome	65 (50 - 76)	70 (50 - 89)	78 (61 - 90)	0.039 (0.035 - 0.044)	25.0 (20.3 - 28.5)	3.93 (3.39 - 4.38)	2.8 (1.6 - 5.5)	8.04 (7.97 - 8.10)	4431 (4187 - 4624)	10.3 (10.1 - 10.7)	1.5 (1.2 - 1.9)	102.3 (95.4 - 107.1)	2.3 (2.2 - 2.3)	13.7 (8.0 - 16.9)	22.6 (13.3 - 32.3)	5.5 (4.4 - 7.0)	2.4 (1.5 - 3.8)	< LD* (< LD* - 0.8)	4.4 (< LD* - 11.8)
10	West Stafford	South Winterbourne	51 (43 - 67)	49 (36 - 62)	53 (39 - 66)	0.036 (0.030 - 0.046)	40.8 (36.9 - 44.9)	3.50 (2.76 - 4.39)	2.1 (1.6 - 2.3)	7.72 (7.63 - 7.78)	4718 (4407 - 4958)	13.7 (13.2 - 14.2)	2.2 (2.2 - 2.4)	113.3 (110.6 - 115.2)	2.6 (2.5 - 2.7)	15.2 (9.4 - 19.1)	7.2 (3.3 - 10.5)	4.0 (1.9 - 6.4)	3.9 (1.9 - 4.8)	< LD* (< LD* - 0.5)	< LD* (< LD* - 2.6)
11	Woodsford	Frome	57 (45 - 70)	56 (43 - 73)	71 (54 - 83)	0.046 (0.033 - 0.063)	30.8 (27.1 - 33.3)	3.79 (3.34 - 4.47)	2.5 (2.0 - 3.4)	7.93 (7.84 - 8.11)	4432 (4318 - 4685)	11.9 (11.5 - 13.0)	1.8 (1.6 - 2.0)	104.6 (100.9 - 107.8)	2.4 (2.3 - 2.4)	15.0 (8.9 - 18.8)	13.5 (9.7 - 20.5)	4.8 (3.9 - 6.1)	2.4 (1.3 - 3.0)	0.5 (< LD* - 1.1)	< LD* (< LD* - 4.2)
12	Moreton Ford	Frome	53 (41 - 68)	53 (43 - 73)	72 (67 - 83)	0.045 (0.035 - 0.064)	29.3 (25.4 - 31.6)	3.81 (3.21 - 4.51)	2.7 (2.1 - 4.3)	8.02 (7.90 - 8.17)	4394 (4210 - 4670)	11.9 (11.2 - 12.9)	1.9 (1.6 - 2.4)	103.5 (99.3 - 106.5)	2.4 (2.4 - 2.5)	15.0 (8.8 - 18.5)	24.8 (13.5 - 49.7)	7.2 (6.2 - 8.0)	4.2 (2.0 - 9.3)	1.1 (< LD* - 2.2)	4.3 (< LD* - 17.6)
13	Broomhills	Tadnoll Brook	30 (24 - 38)	31 (27 - 41)	72 (31 - 94)	0.067 (0.034 - 0.084)	30.2 (24.3 - 33.5)	3.83 (3.55 - 4.12)	5.1 (2.6 - 10.1)	7.84 (7.69 - 8.05)	3865 (3352 - 4470)	15.7 (15.3 - 15.9)	2.8 (2.1 - 3.9)	93.4 (81.0 - 104.2)	3.4 (3.3 - 3.7)	19.6 (14.3 - 23.4)	175.5 68.3 - 386.3)	26.8 (16.6 - 31.6)	10.1 (7.4 - 14.4)	0.8 (< LD* - 1.9)	37.6 (15.6 - 66.2)
14	East Stoke	Frome	42 (25 - 54)	45 (34 - 53)	67 (61 - 73)	0.037 (0.016 - 0.046)	27.4 (23.2 - 29.7)	3.69 (2.82 - 4.43)	4.2 (2.3 - 9.3)	8.02 (7.91 - 8.16)	4334 (3944 - 4634)	12.6 (11.9 - 13.2)	2.1 (1.8 - 2.6)	98.9 (92.0 - 104.2)	2.6 (2.5 - 2.7)	15.7 (10.0 - 19.1)	50.6 (24.0 - 110.7)	13.3 (9.8 - 14.5)	4.3 (2.5 - 7.5)	0.5 (< LD* - 1.1)	10.4 (2.8 - 15.8)

Sites 8 and 10 - n = 4, all others - n = 5

* < LD = below limit of detection